



US008980851B2

(12) **United States Patent**  
**O'Connor**

(10) **Patent No.:** **US 8,980,851 B2**  
(45) **Date of Patent:** **\*Mar. 17, 2015**

(54) **METHODS FOR BONE TREATMENT BY  
MODULATING AN ARACHIDONIC ACID  
METABOLIC OR SIGNALING PATHWAY**

FOREIGN PATENT DOCUMENTS

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(\* ) Notice: Subject to any disclaimer, the term of this  
patent is extended or adjusted under 35  
U.S.C. 154(b) by 0 days.

This patent is subject to a terminal dis-  
claimer.

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(21) Appl. No.: **12/940,995**

(22) Filed: **Nov. 5, 2010**

(65) **Prior Publication Data**

US 2011/0124717 A1 May 26, 2011

**Related U.S. Application Data**

(63) Continuation of application No. 11/995,529, filed as  
application No. PCT/US2006/032367 on Aug. 18,  
2006, now Pat. No. 7,829,535.

(60) Provisional application No. 60/709,838, filed on Aug.  
18, 2005.

(51) **Int. Cl.**

**A61K 48/00** (2006.01)  
**A61K 31/12** (2006.01)  
**A61K 31/05** (2006.01)  
**A61K 31/405** (2006.01)

(52) **U.S. Cl.**

CPC ..... **A61K 31/405** (2013.01); **A61K 48/00**  
(2013.01); **A61K 31/12** (2013.01); **A61K 31/05**  
(2013.01)

USPC ..... **514/44 R**; 514/688; 514/734

(58) **Field of Classification Search**

CPC ..... **A61K 48/00**; **A61K 31/12**; **A61K 31/05**  
USPC ..... **514/44 R**, 688, 734  
See application file for complete search history.

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(57) **ABSTRACT**

Methods for promoting osteogenesis to accelerate or enhance  
bone fracture healing, treat bone defects, and enhance bone  
formation are disclosed. The methods modulate an arachi-  
donic acid metabolic or signaling pathway in general, and, in  
particular, utilize 5-lipoxygenase inhibitors. These molecules  
can be delivered alone or in combination with one or more  
agents that inhibit bone resorption, regulate calcium resorp-  
tion from bone, enhance bone accumulation, enhance bone  
formation, induce bone formation, impair growth of micro-  
organisms, reduce inflammation, and/or reduce pain.

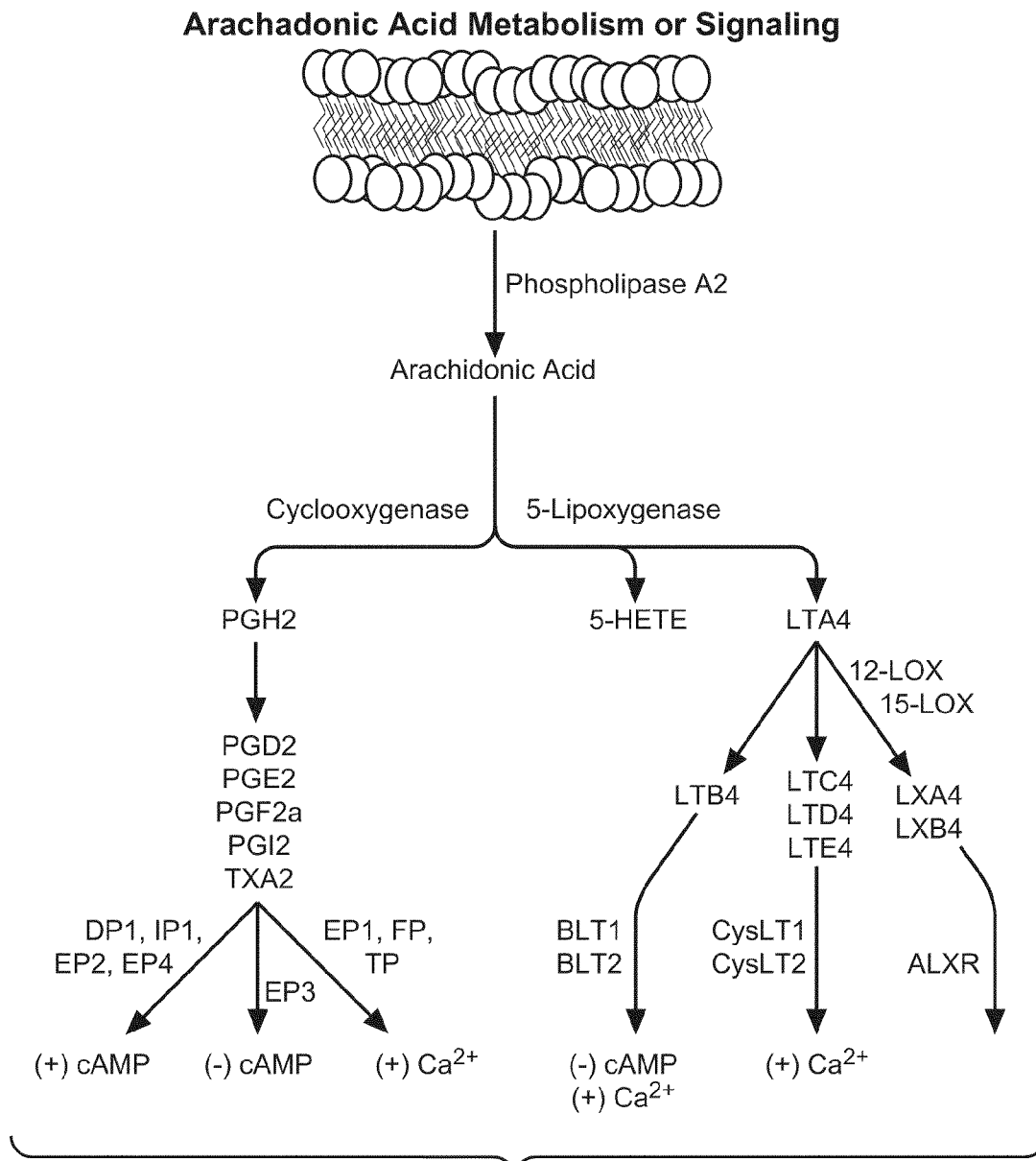
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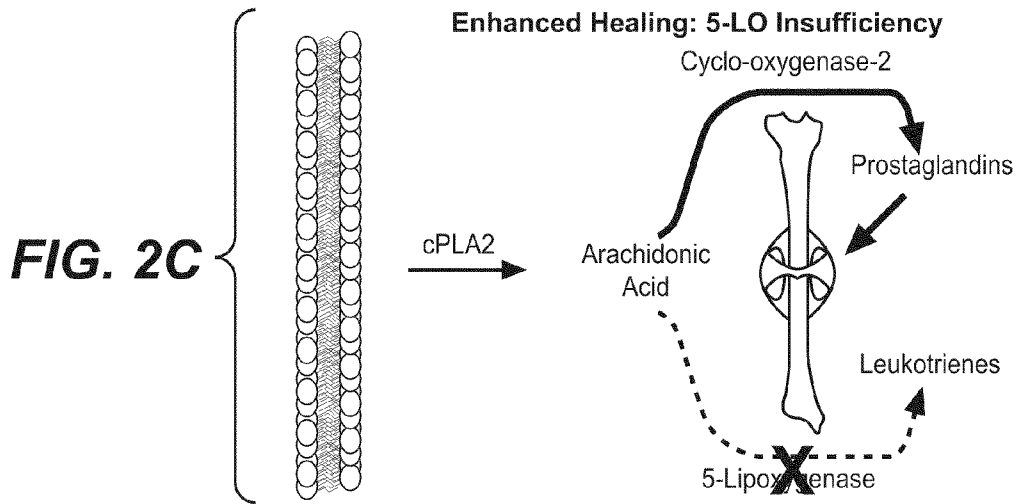
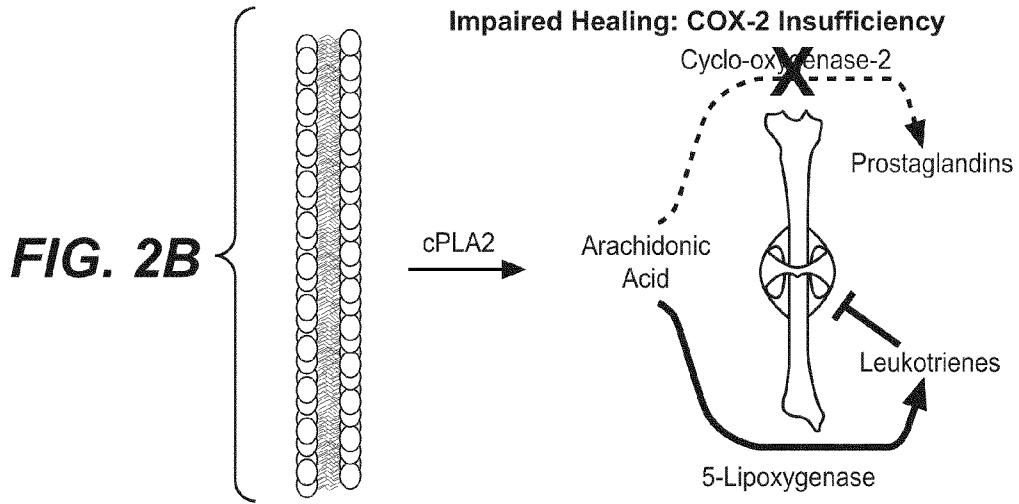
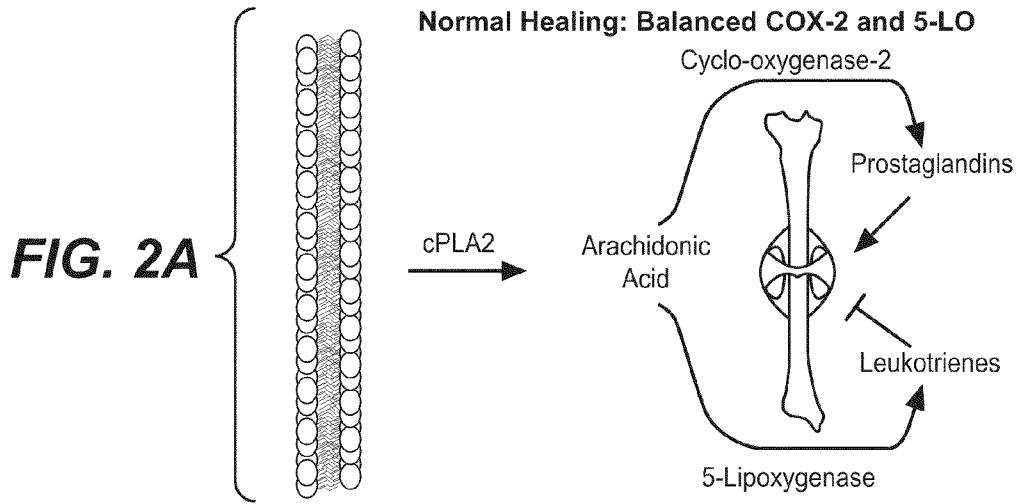
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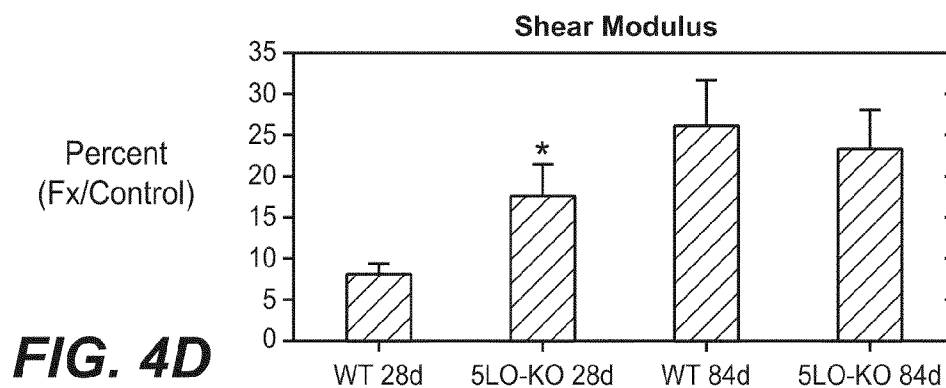
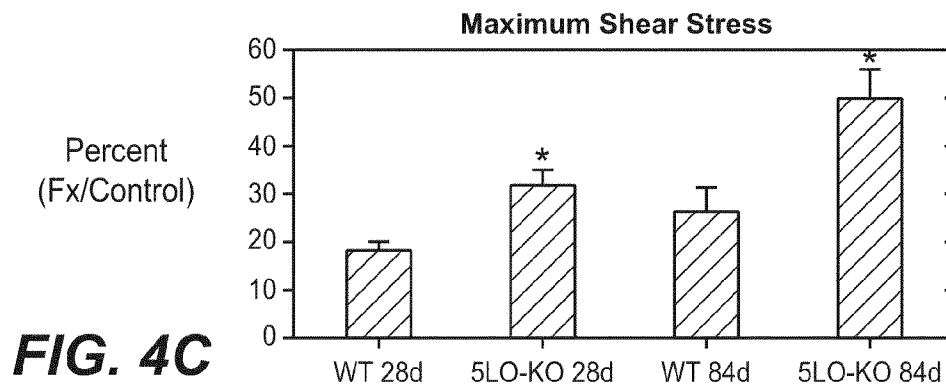
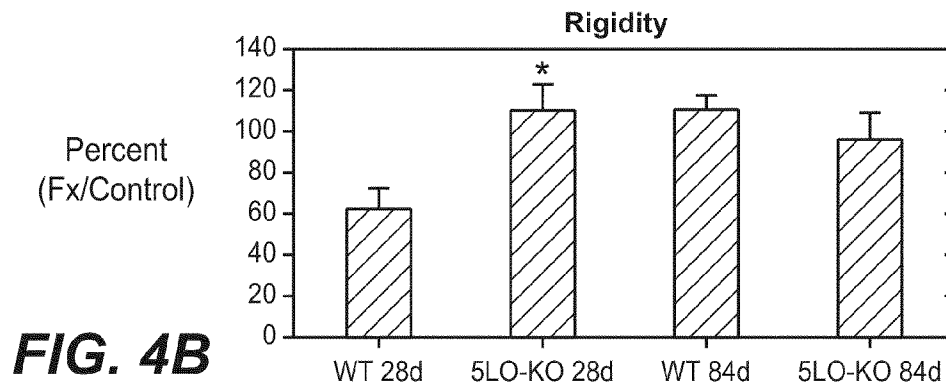
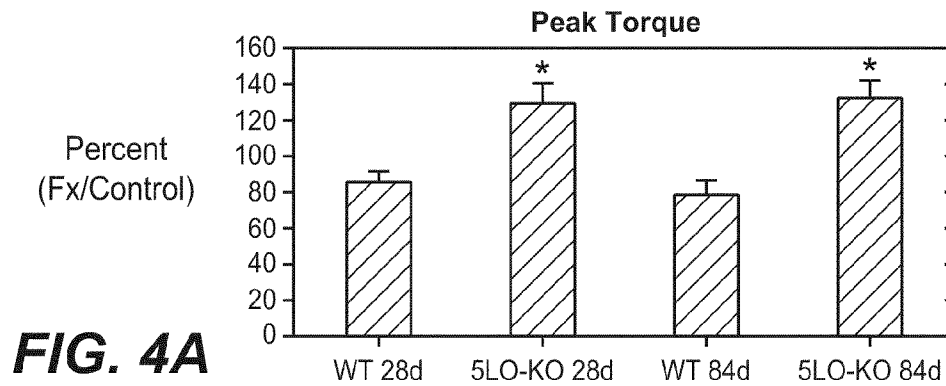


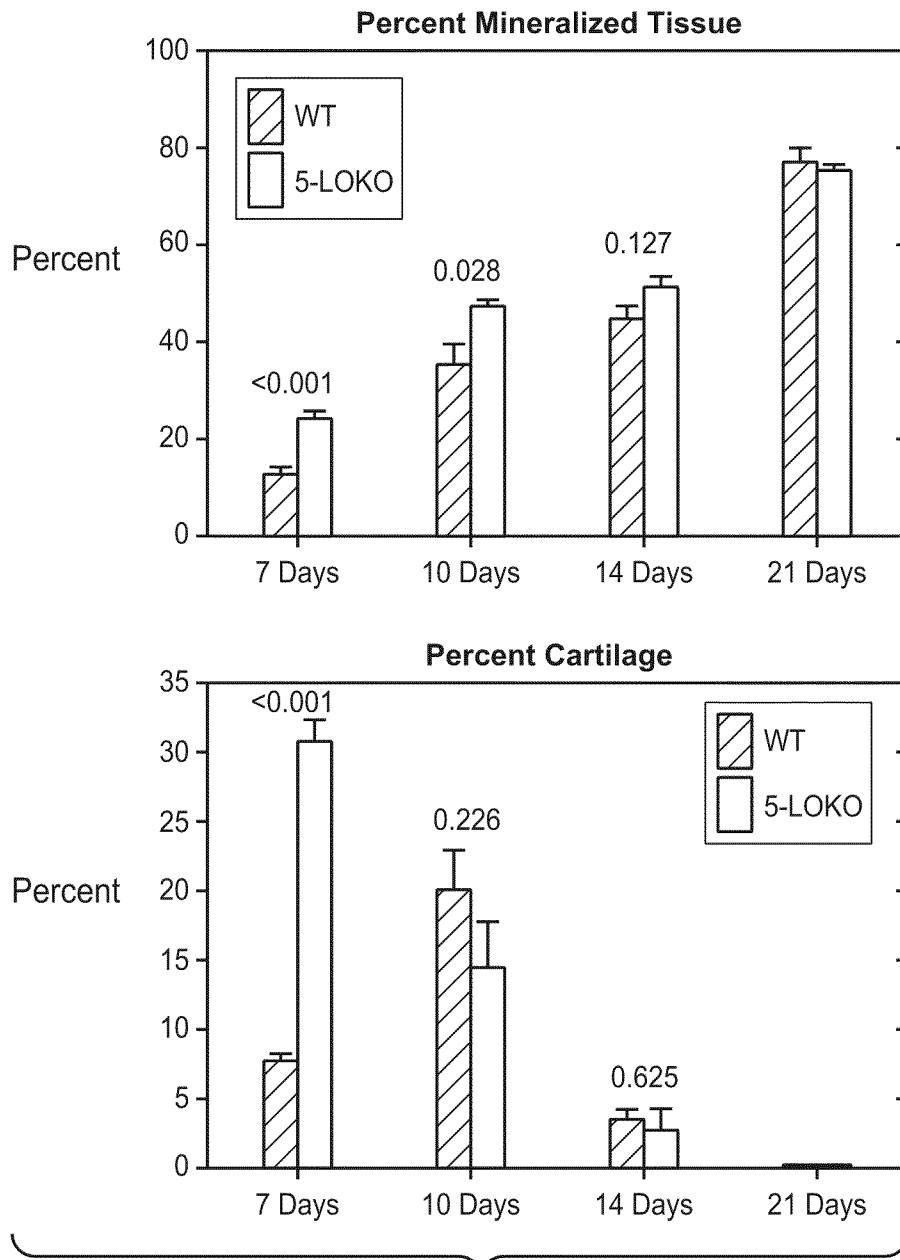
**FIG. 1**





**FIG. 3**





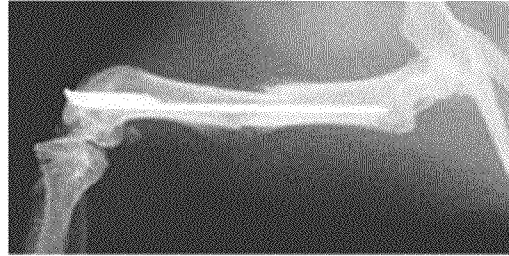
**FIG. 5**

*Cox1<sup>-/-</sup>*



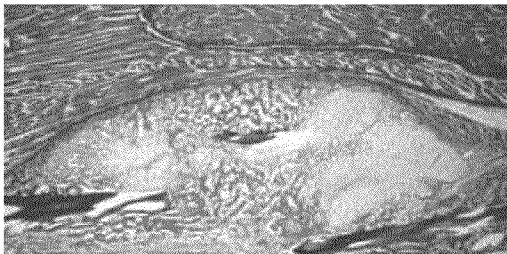
**FIG. 6A**

*Cox2<sup>-/-</sup>*



**FIG. 6D**

*Cox1<sup>-/-</sup>* (5X)



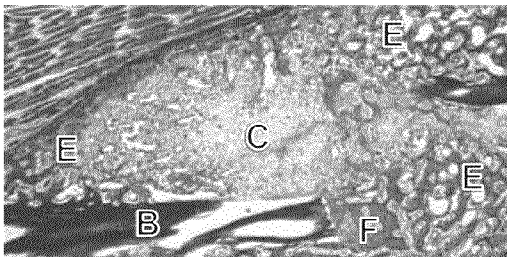
**FIG. 6B**

*Cox2<sup>-/-</sup>* (10X)



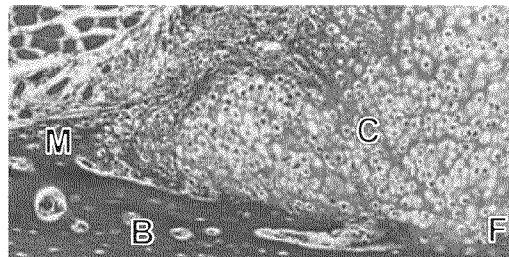
**FIG. 6E**

*Cox1<sup>-/-</sup>* (10X)

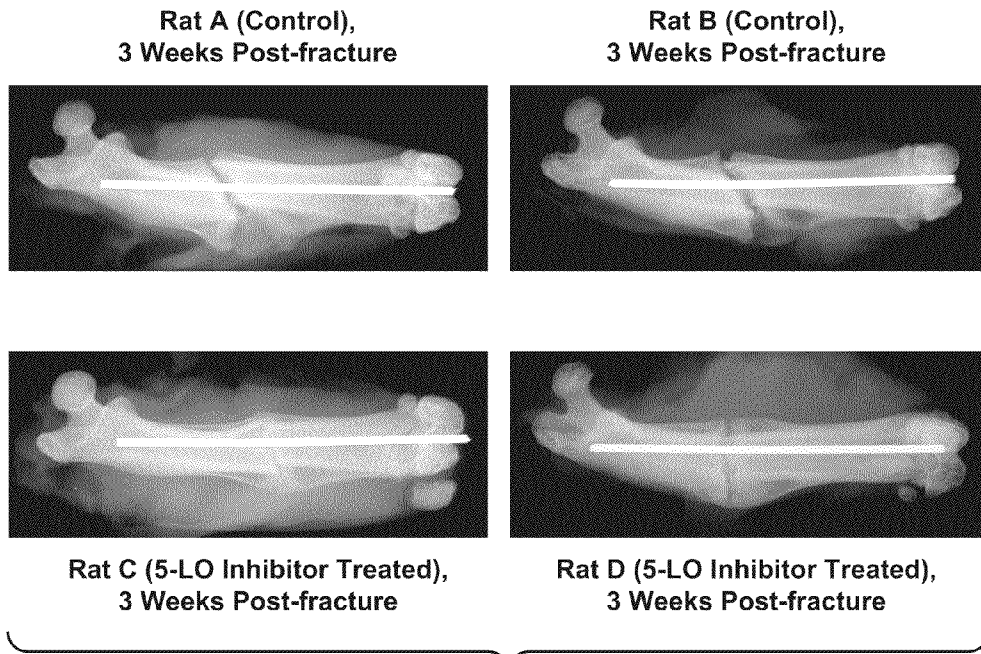


**FIG. 6C**

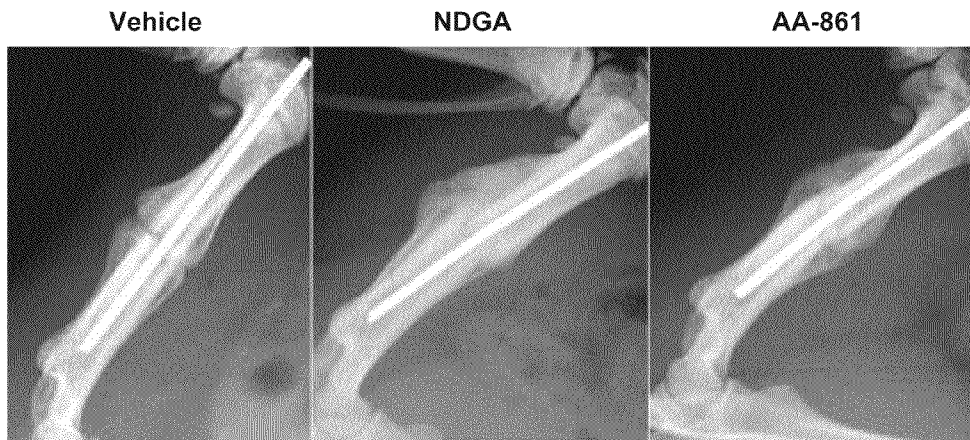
*Cox2<sup>-/-</sup>* (25X)



**FIG. 6F**



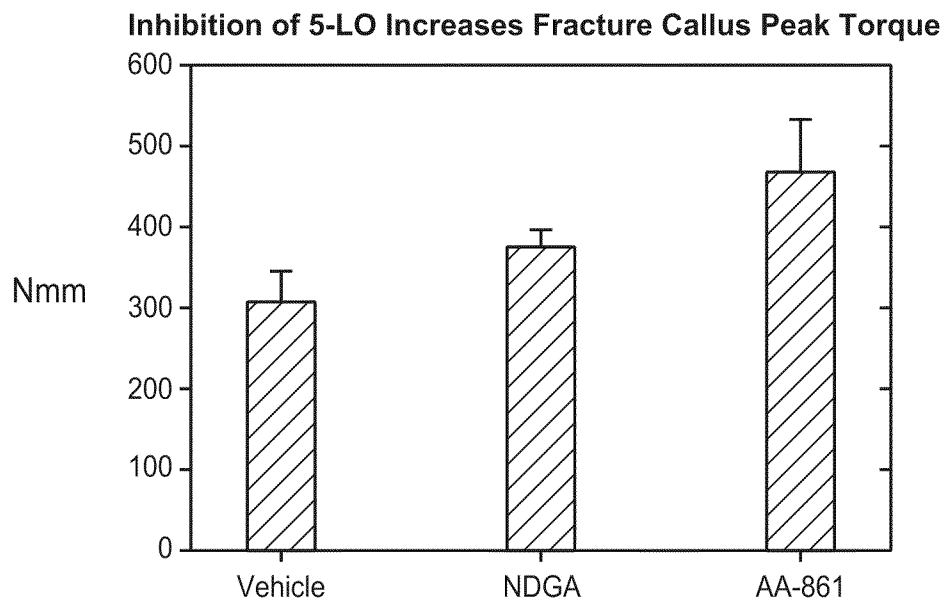
**FIG. 7**



**FIG. 8A**

**FIG. 8B**

**FIG. 8C**



**FIG. 8D**

## METHODS FOR BONE TREATMENT BY MODULATING AN ARACHIDONIC ACID METABOLIC OR SIGNALING PATHWAY

### CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. patent application Ser. No. 11/995,529, granted as U.S. Pat. No. 7,829,535, which is a National Stage of International Application No. PCT/US2006/032367, filed Aug. 18, 2006, published in English under PCT Article 21(2), which claims the benefit of U.S. Provisional Application Ser. No. 60/709,838, filed on Aug. 18, 2005, each of which are incorporated by reference in their entirety.

### SEQUENCE LISTING

This application includes a Sequence Listing submitted electronically as a text file named "17738US\_seqencelisting.txt," created on Nov. 5, 2010, with a size of 42 kb. The sequence listing consists of 43 sequences and is incorporated by reference.

### FIELD OF INVENTION

The invention relates generally to accelerating or enhancing bone formation or fracture healing by modulating an arachidonic acid metabolic or signaling pathway, in particular by using inhibitors of 5-lipoxygenase activity.

### BACKGROUND OF THE INVENTION

Bone fractures are a common traumatic injury. Approximately 8-10 million bone fractures are reported annually in the United States with more than 1 million of these requiring hospitalization. The estimated annual costs of treating these fractures exceeds 20 billion dollars. While this is already significant, these numbers are expected to increase due to the aging of the general population. Further, among military personnel, bone fractures are common training injuries. Bone fractures, typically located in the arms and legs, are also common battle wounds. Aside from traumatic injury, bone fractures also can be caused by disease. Osteoporosis is caused by a reduction in bone mineral density in mature bone and results in fractures after minimal trauma. The disease is widespread and has a tremendous economic impact. The most common fractures occur in the vertebrae, distal radius and hip. An estimated one-third of the female population over age 65 will have vertebral fractures, caused in part by osteoporosis. Moreover, hip fractures are likely to occur in about one in every three woman and one in every six men by extreme old age.

Fracture healing is a complex tissue regeneration process that involves cell migration, proliferation, apoptosis, and differentiation in response to growth factors, cytokines, other signaling molecules, and to the mechanical environment. The temporal order and magnitude of each cellular process must be controlled for optimal regeneration. The normal events of fracture healing are described below as occurring in 4 phases. In the initial phase, hematoma formation and localized tissue hypoxia are the initial cellular and molecular events of fracture healing. The second phase, called the early stage, is characterized by inflammation followed by rapid accumulation of cells at the fracture site. The presence of macrophages and neutrophils at the fracture site during inflammation precedes the rapid migration and proliferation of mesenchymal

cells at the fracture site. In the third, regenerative phase, endochondral ossification creates the new bone which bridges the fracture. At this point, the fracture callus has a well-defined morphology. Intramembraneous ossification creates buttresses of periosteal bone at the callus periphery. Mesenchymal cells within the callus begin to differentiate into chondrocytes at the interface of the periosteal bone buttress. Each new chondrocyte develops as would be expected with matrix deposition followed by matrix calcification to produce calcified cartilage and then apoptosis. Channels are formed into the calcified cartilage starting at the periosteal bone buttresses. Osteoblasts migrate or differentiate on the surface of the calcified cartilage within these channels and begin depositing new bone. As chondrocyte differentiation proceeds from the periphery to the center of the callus (fracture site), channel formation, osteoblast differentiation, and new bone formation follows until the soft callus has been replaced with woven (immature) bone. Angiogenesis during the regenerative phase is essential. The immature woven bone created during the regenerative phase is mechanically unsuited for normal weight-bearing. To compensate for the decreased mechanical properties of the woven bone, the fracture callus has a significantly larger diameter which provides for greater structural mechanical properties. In the final, remodeling phase, fracture callus diameter diminishes until the bone obtains its normal dimensions while maintaining the bones overall mechanical properties by enhancing material mechanical properties. This is accomplished by replacing the mechanically poor, woven bone with mechanically strong, lamellar (mature) bone. In successive rounds, osteoclasts resorb the woven bone and osteoblasts replace it with lamellar bone. Molecular mechanisms governing osteoclast formation and function occurs through the RANKL-RANK pathway and this pathway is activated during fracture healing.

Fractures are generally treated conservatively by closed reduction of the fracture and immobilization (casting) of the affected bone. In such cases, the bone heals through the endochondral ossification pathway described above. Adequate nutrition to include vitamin C, vitamin D, and calcium aids in healing. There has been no major advancement in the treatment of bone fractures since the mid 20<sup>th</sup> century when open reduction and internal fixation of fractures became commonplace. The promise of growth factor treatments to enhance fracture healing has not been realized yet.

Unfortunately, many fractures require surgical intervention to increase healing success and reduce the likelihood of complication. There is only one approved pharmacological enhancement for bone healing and that is treatment with recombinant bone morphogenetic protein, either BMP-2 or BMP-7 (OP-1). Use of these growth factors requires surgery and due to expense and unknown potential side effects caused by the use of supraphysiological levels of growth factors, BMPs are used as a last-resort to heal recalcitrant fractures. Typical patient care also involves the administration of antibiotics, a narcotic, an NSAID, a COX-2 inhibitor or other pain killers during the healing process.

NSAIDs inhibit cyclooxygenase, thereby inhibiting the conversion of arachidonic acid into prostaglandins (PGD<sub>2</sub>, PGE<sub>2</sub>, PGF<sub>2</sub>α, PGI<sub>2</sub>, TXA<sub>2</sub>). Arachidonic acid is also a precursor for the leukotrienes (LTB<sub>4</sub>, LTC<sub>4</sub>, LTD<sub>4</sub>, LTE<sub>4</sub>), lipoxins (LXA<sub>4</sub>, LXB<sub>4</sub>), and 5-hydroxyeicosatetraenoic acid (5-HETE). The enzyme 5-lipoxygenase (5-LO) converts arachidonic acid to 5-hydroperoxyeicosatetraenoic acid (5-HpETE). This is the first step in the metabolic pathway which yields 5-HETE, the leukotrienes (LTs), and the lipoxins. Leukotrienes are also pro-inflammatory with the

ability to attract neutrophils and cause capillary permeability. The arachidonic acid metabolic pathway is summarized in FIG. 1.

Lipoxygenases are nonheme iron-containing enzymes found in plants and animals that catalyze the oxygenation of certain polyunsaturated fatty acids, such as lipids and lipoproteins. Several lipoxygenase enzymes are known, each having a characteristic oxidation action. Mammalian lipoxygenases are named by the position in arachidonic acid that is oxygenated. For example, the enzyme 5-lipoxygenase converts arachidonic acid to 5-hydroperoxyeicosatetraenoic acid (5-HpETE), while the enzyme 12-lipoxygenase converts arachidonic acid to 12-HpETE. The activity of 5-lipoxygenase requires a co-factor commonly called FLAP (five lipoxygenase activating protein). Leukotriene synthesis is reduced by drugs that inhibit FLAP (MK866) or mice lacking FLAP.

WO 95/30419 discloses 5-LO inhibitors reduce osteoclast activity. The suppression of osteoclast activity inhibits bone resorption and reduces bone loss in human pathological conditions. Bone resorption is an integral part of fracture healing because it is necessary to remodel the newly formed bone into stronger, more mature bone. The inhibition of bone resorption would be expected to impair the later stages of normal fracture healing. Koivukangas et al., *Long-term administration of clodronate does not prevent fracture healing in rats*. Clinical Orthopaedics and Related Research 408: 268-278 (2003) and Peter et al. *Effect of alendronate on fracture healing and bone remodeling in dogs*. Journal of Orthopaedic Research 14: 74-79 (1996) disclose the effects of bisphosphonate therapy on fracture healing. The data show that bisphosphonate therapy which impairs osteoclast activity and bone remodeling does not inhibit the initial stages of fracture repair but does impair the later bone remodeling stage. The bisphosphonate effect on fracture healing reveals itself as persistence of a large fracture callus that contains mechanically immature, woven bone rather than mechanically mature, lamellar bone.

WO 03/066048 discloses that 12/15-lipoxygenase inhibitors can be used to prevent bone loss or increase bone mass. The publication describes data showing that bone mineral density is preserved in transgenic mouse that overexpress IL-4 and that were treated with a 15-LO inhibitor. The publication does not disclose that 15-LO inhibitors can aid fracture healing or the treatment of non-unions.

Traianedes, K., et al., *5-Lipoxygenase metabolites inhibit bone formation in vitro*. Endocrinology, 139: 3178-3184 (1998) discloses the effects of LTB<sub>4</sub>, 5-HETE, and LTD<sub>4</sub> (all products of 5-LO function) on the differentiation of fetal rat calvaria (osteoblast) cells. The data show that 5-HETE and LTB<sub>4</sub> reduce bone nodule formation and alkaline phosphatase activity in vitro but that LTD<sub>4</sub> had no effect. The results from an in vitro organ culture model showed that LTB<sub>4</sub> or 5-HETE treatment prevented a BMP2 induced increase in mouse calvaria thickness. The publication, however, does not disclose the use of any 5-LO inhibitors, nor does it disclose that 5-LO inhibition would lead to the same effect in cultured osteoblasts or in organ cultures. Similarly, Ren and Dziak, *Effects of leukotrienes on osteoblast cell proliferation*. Calcified Tissue International 49: 197-201 (1991) discloses that LTB<sub>4</sub> treatment reduces proliferation of primary rat calvaria (osteoblast) cultures in vitro, but that LTB<sub>4</sub> can promote proliferation of established osteoblast cell lines (Saos-2 and G292) in vitro at higher concentration (0.3-1 micromolar). Ren and Dziak also disclose that LTC<sub>4</sub> had no effect on the proliferation of primary rat osteoblast cells or Saos-2 cells but did promote proliferation of G292 cells. Further, Ren and Dziak disclose that treatment of

Saos-2 cells with a 5-LO inhibitor (AA-861) had no effect on Saos-2 cell proliferation. The publication indicates that 5-LO inhibition should have no effect on osteogenesis.

Thus, it is readily apparent that compositions and methods for accelerating or enhancing bone formation or fracture healing would be highly desirable.

## SUMMARY

The present invention provides methods of promoting osteogenesis by administering a compound that reduces a 5-lipoxygenase activity to treat a bone fracture, a bone defect or a condition treated by inducing bone formation.

In another aspect of the invention, the methods can further comprise an additional active agent such as a modulator of the activity of a cyclooxygenase. In one aspect the activity of a cyclooxygenase-2 (COX-2) is increased. In another aspect, the activity of cyclooxygenase-1 (COX-1) is reduced.

In one aspect, the methods use in vivo administration of a compound. In another aspect, ex vivo administration of a compound is used.

In one aspect, the compound is a small molecule. In another aspect the compound is an antisense compound. In another aspect, the compound is an RNAi compound.

These and other aspects of the present invention will become evident upon reference to the following detailed description and attached figures. In addition, various references are set forth herein which describe in more detail certain procedures or compositions, and are therefore incorporated by reference in their entirety.

## BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 summarizes an exemplary arachidonic acid metabolic or signaling pathway.

FIG. 2 illustrates the modulation of arachidonic acid metabolism by altering cyclooxygenase activity or lipoxygenase activity to accelerate or enhance bone formation. FIG. 2A represents the normal functioning of the pathway. FIG. 2B shows that the inhibition of COX-2 activity leads to excess leukotriene production which impairs bone formation in fracture healing or other osteogenic processes. FIG. 2C shows that the inhibition of lipoxygenase activity leads to excess prostaglandin production which accelerates or enhances bone formation in fracture repair or other osteogenic processes.

FIG. 3 shows that serial x-rays of femur fractures made from a 5LO<sup>-/-</sup> mouse and a normal mouse (C57BL/6). The x-rays show that osteogenesis, and therefore fracture healing is accelerated in the 5LO<sup>-/-</sup> mouse.

FIG. 4 illustrates mechanical testing data of fracture healing in wild-type (WT) and 5-LO knockout mice (5LO-KO or 5-LO<sup>-/-</sup>) 28 days and 84 days after the onset of the fracture. Peak torque (FIG. 4A), rigidity (FIG. 4B), maximum shear stress (FIG. 4C), and shear modulus (FIG. 4D) were calculated from callus dimensions and the torque to angular displacement curves.

FIG. 5 illustrates histomorphometric data of fracture healing from wild-type (WT) and 5-LO knockout mice (5-LOKO or 5-LO<sup>-/-</sup>) at 7, 10, 14, and 21 days after fracture. The left panel shows the percent of fracture callus area that is newly formed bone (mineralized tissue) and the right panel shows the percent of fracture callus area that is cartilage.

FIG. 6 shows that fracture healing is dramatically impaired in COX-2 knock-out mice and that the defect in healing occurs because of lack of osteogenesis (new bone formation). FIG. 6A shows data from x-rays and FIGS. 6B and 6C show the histological samples of 14-day old femur fractures in mice

lacking a functional COX-1 gene. FIG. 6D shows data from x-rays and FIGS. 6E and 6F show the histological samples of 14-day old femur fractures in mice lacking a functional COX-2 gene.

FIG. 7 illustrates that osteogenesis is accelerated in rats treated with 5-LO inhibitors, resulting in fractures healing faster than in untreated rats.

FIG. 8 illustrates that osteogenesis is accelerated in rats treated with two different 5-LO inhibitors, resulting in fractures healing faster than in untreated rats. FIGS. 8A, 8B, and 8C show data from x-rays for vehicle control (8A), NDGA (8B), and AA-861 (8C). FIG. 8D is a graph showing inhibition of 5-LO increases fracture callus peak torque.

#### DETAILED DESCRIPTION OF THE INVENTION

The practice of the present invention will employ, unless otherwise indicated, conventional methods of protein chemistry, biochemistry, recombinant DNA techniques and pharmacology, within the skill of the art. Such techniques are explained fully in the literature. See, e.g., T. E. Creighton, *Proteins: Structures and Molecular Properties* (W.H. Freeman and Company, 1993); A. L. Lehninger, *Biochemistry* (Worth Publishers, Inc., current addition); Sambrook, et al., *Molecular Cloning: A Laboratory Manual* (2nd Edition, 1989); *Methods In Enzymology* (S. Colowick and N. Kaplan eds., Academic Press, Inc.); *Remington's Pharmaceutical Sciences*, 18th Edition (Easton, Pa.: Mack Publishing Company, 1990); Carey and Sundberg *Advanced Organic Chemistry 3<sup>rd</sup> Ed.* (Plenum Press) Vols A and B (1992).

All publications, patents and patent applications cited herein, whether supra or infra, are hereby incorporated by reference in their entirety.

#### I. DEFINITIONS

In describing the present invention, the following terms will be employed, and are intended to be defined as indicated below.

By "modulating an arachidonic acid metabolic or signaling pathway" is meant use of a drug or a compound which inhibits or promotes the activity or concentration of any enzyme or regulatory molecule involved in an arachidonic acid metabolism or signal pathway in a cell or animal. Preferably drug or a compound can be selected from a FLAP inhibitor such as BAYx 1005, MK-886, and MK-0591; a 5-Lipoxygenase inhibitor such as Zileuton, BAY-G576, RS-43,179, Wy-47, 288, ABT-761, vitamin A, and BW A4C; leukotriene receptor antagonists such as zafirlukast, montelukast, pranlukast, ICI-204,219, MK-571, MK-679, ONO-RS-411, SK&F 104,353, and Wy-48,252; a leukotriene B4 receptor antagonists; a leukotriene C4 synthase inhibitors; a Leukotriene A4 hydrolase inhibitors; a non-steroidal antiinflammatory drug (NSAID), a leukotriene receptor antagonists and leukotriene analogs, compounds modulating the formation and action of leukotrienes, compounds that affect cyclooxygenase activity, compounds that affect prostaglandin activity such as receptor agonists or antagonists, prostaglandin analogs, compounds that affect leukotriene activity such as receptor agonists or antagonists, and leukotriene analogs.

By "accelerated" is meant that osteogenesis occurs more rapidly and the time required for bone healing is reduced, or the bone heals more quickly in a treated subject as compared to an untreated subject or a control subject.

By "enhancing" is meant that the healed bone in the treated subject has improved characteristics compared to an untreated subject, or a control subject such as, for example, greater bone strength.

By "fracture healing" or "fracture repair" is meant that, in particular, promoting the healing of bone fractures and bone defects, and improving the mechanical stability of the healing fracture or site. Such bone fractures may be, for example, the common, traumatic (disabling and non-osteoporotic) fractures, the osteoporotic fractures due to osteoporosis or osteopenia of any etiology, fractures due to Paget's disease or fractures due to bone loss as a consequence of side effects of other drugs, e.g. in patients receiving high doses of corticosteroids, fractures arising from other congenital or acquired disease such as, e.g., osteogenesis imperfecta and breast cancer, surgical created fractures (osteotomies) used for example in bone lengthening and limb lengthening procedures, and treatment of bone fracture delayed unions or non-unions. The invention augments fracture healing following normal reduction and immobilization of the fracture using techniques common to one skilled in the art by accelerating and enhancing bone formation.

By "bone formation" is meant that the rate of bone formation in a subject treated according to the methods of the invention, such as, e.g., by receiving a 5-lipoxygenase inhibitor, is increased over the bone formation rate in a subject that is not given a 5-lipoxygenase inhibitor. Such enhanced bone formation is determined herein using, e.g., quantitative digitized morphometry, as well as by other markers of bone formation, as described above. Bone formation is meant to include the osteogenic process used for spine fusions and other joint or bone ankylosis application, bone formation into or around prosthetic devices, or bone formation to augment existing bones or replace missing bones or bone segments.

By "osteogenesis" is meant the production of bone that is associated with repair of a fractured bone, repair of a bone that has a defect caused by intentional or non-intentional damage, or induction of bone formation used to fuse more than one bone or bone segment together. "Osteogenesis" is not meant to include bone formation associated with normal bone growth in adolescents. "Osteogenesis" also is not meant to include bone formation associated with normal bone homeostasis, which is often referred to as bone remodeling, in which bone is normally turned-over by a process whereby osteoclasts resorb bone and osteoblasts make new bone to replace that which has been resorbed.

By "bone defect" is meant damage to a bone such that a portion of the bone is removed or is otherwise missing. Such bone defects would include anomalous holes, gaps or openings created in the bone for purposes of a diagnostic or therapeutic procedure, loss of bone segments from trauma or disease, puncture wounds to the bone, and the like.

The term "modulating" refers to the effect of a modulator on an arachidonic acid metabolic or signaling pathway. A modulator can be, e.g., a polypeptide, nucleic acid, macromolecule, complex molecule, small molecule, compound, or the like (naturally occurring or non-naturally occurring) that is capable of causing modulation. Modulators can be evaluated for potential activity as inhibitors or activators (directly or indirectly) of a functional property, biological activity or process, or a combination thereof (e.g., agonist, partial antagonist, partial agonist, inverse agonist, antagonist, and the like), by inclusion in assays that measure the activity of an enzyme in the pathway.

The terms "effective amount" or "pharmaceutically effective amount" refer to a sufficient amount of an agent to provide the desired biological result. That result can be reduction and/or alleviation of the signs, symptoms, or causes of a disease, or any other desired alteration of a biological system. For example, an "effective amount" for therapeutic uses is the amount of the composition comprising an active compound

herein required to provide a clinically significant increase in osteogenesis and, thus, healing rates in fracture repair; reversal of cartilage defects or disorders; stimulation and/or augmentation of bone formation in fracture non-unions, delayed unions and distraction osteogenesis; increase and/or acceleration of bone growth into prosthetic devices; enhanced or accelerated bone formation in joint ankylosis, bone ankylosis, or spine fusions, bone formation to augment existing bone or replace missing bone or bone segments such as during autograft, allograft, or synthetic bone material incorporation, and repair of dental defects.

As used herein, the terms “treat” or “treatment” are used interchangeably and are meant to indicate administering one or more compounds in accordance with the methods of the invention to promote osteogenesis to obtain a desired therapeutic objective. The terms further include ameliorating existing bone or cartilage deficit symptoms, preventing additional symptoms, ameliorating or preventing the underlying metabolic causes of symptoms, and/or encouraging bone growth.

As used herein, “small molecule” is meant to indicate a chemical compound having a molecular weight of less than about 500 daltons. Small molecules do not include biologic polymers such as polypeptides and polynucleotides.

By “pharmaceutically acceptable” or “pharmacologically acceptable” is meant a material which is not biologically or otherwise undesirable, i.e., the material may be administered to an individual without causing any undesirable biological effects or interacting in a deleterious manner with any of the components of the composition in which it is contained.

By “physiological pH” or a “pH in the physiological range” is meant a pH in the range of approximately 7.2 to 8.0 inclusive, more typically in the range of approximately 7.2 to 7.6 inclusive.

As used herein, the term “subject” encompasses mammals. Examples of mammals include, but are not limited to, any member of the Mammalia class: humans, non-human primates such as chimpanzees, and other apes and monkey species; farm animals such as cattle, horses, sheep, goats, swine; domestic animals such as rabbits, dogs, and cats; laboratory animals including rodents, such as rats, mice and guinea pigs, and the like. The term does not denote a particular age or gender.

The compounds of the present invention may be used to inhibit or reduce the activity of 5-lipoxygenase, 5-lipoxygenase and cyclooxygenase, and other enzymes and compounds in an arachadonic acid metabolic or signaling pathway. In this context, inhibition and reduction of the enzyme activity refers

to a lower level of measured activity relative to a control experiment in which the enzyme, cell, or subject is not treated with the test compound. In particular embodiments, the inhibition or reduction in the measured activity is at least a 10% reduction or inhibition. One of skill in the art will appreciate that reduction or inhibition of the measured activity of at least 20%, 50%, 75%, 90% or 100% or any amount between 10% and 100%, may be preferred for particular applications. Inhibition of enzyme activity may be through any mechanism, including, by way of example, but not limitation, a reduction in the amount of enzyme present, a competitive or non-competitive inhibition of catalytic activity, an interference with an interaction between the enzyme and a co-factor or accessory protein, etc. In addition, the compounds of the present invention may be used to increase a COX-2 activity. In particular embodiments, the increase of enzyme activity refers to a higher level of measured activity relative to a control experiment in which the enzyme, cell, or subject is not treated with the test compound. In particular embodiments, the increase in measured activity is at least a 10% increase. One of skill in the art will appreciate that an increase of the measured activity of at least 20%, 50%, 75%, 90% or 100% or any amount between 10% and 100% or beyond, may be preferred for particular applications. Increase of enzyme activity may be through any mechanism, including, by way of example but not limitation, an increase in the amount of enzyme present, or by increasing the enzyme’s turnover rate, or altering its substrate binding properties.

References to the enzymes 5-lipoxygenase (5-LO), COX-1, and COX-2 are intended to encompass the exemplary sequences referenced in Table 1, some of which are provided immediately following Table 1, as well as sequences at least 90% identical, or at least 95%, or at least 96%, or at least 97%, or at least 98%, or at least 99% identical to the exemplary sequences as can be ascertained by one of ordinary skill using routine alignment algorithms such as e.g., BLAST. In addition, other mammalian homologues are encompassed. Such homologues are identified as such on the basis of e.g., sequence similarity, functional similarity, and by chromosome location. In addition to protein sequence, exemplary nucleic acid sequences are provided from which one of ordinary skill can readily obtain sequences of anti-sense and RNAi compounds useful for inhibiting the activity of the enzyme in accordance with the methods of the invention. Anti-sense compounds useful for practice of the invention are known in the art and can be obtained through commercial sources, as described in, e.g., Ding et al. (1999) BBRC Vol. 261, pp. 218-223 (incorporated by reference).

TABLE 1

Exemplary Sequences							
	OMIM	Entrez Gene	GeneBank		Protein	Similarity	
Symbol	ID	ID	Accession Number	mRNA (GenBank)	(Swiss-Prot)	to human sequence	
Name: arachidonate 5-lipoxygenase; aka: 5-LO, 5-lipoxygenase							
Human	ALOX5	152390	240	NC_000010	NM_000698	P09917	NA
Rat	Alox5	NA	25290	NC_005103	NM_012822	P12527	86.29% (n <sup>1</sup> ) 92.94% (p <sup>2</sup> )
Mouse	Alox5	NA	11689	NC_000072	NM_009662	P48999	87.88% (n) 93.47% (p)

TABLE 1-continued

Exemplary Sequences							
Name: arachidonate 5-lipoxygenase-activating protein; aka: FLAP							
Human	ALOX5AP	603700	241	NC_000013	NM_001629	P20292	NA
Rat	Alox5ap	NA	29624	NC_005111	NM_017260	P20291	85.09% (n) 91.93% (p)
Mouse	Alox5ap	NA	11690	NC_000071	NM_009663	P30355	85.71% (n) 91.93% (p)
Name: prostaglandin-endoperoxide synthase 2; aka: cyclooxygenase-2, COX-2, PGHS-2							
Human	PTGS2	600262	5743	NC_000001	NM_000963	P35354	NA
Rat	Ptgs2	NA	29527	NC_005112	NM_017232	P35355	83.17% (n) 84.91% (p)
Mouse	Ptgs2	NA	19225	NC_000067	NM_011198	Q05769	84.71% (n) 86.75% (p)
Name: prostaglandin-endoperoxide synthase 1; aka: cyclooxygenase-1, COX-1, PGHS-1							
Human	PTGS1	176805	5742	NC_000009	NM_000962	P23219	NA
Rat	Ptgs1	NA	24693	NC_005102	NM_017043	Q63921	84.87% (n) 88.11% (p)
Mouse	Ptgs1	NA	19224	NC_000068	NM_008969	P22437	85.59% (n) 89.78% (p)

## Human 5-Lipoxygenase mRNA Sequence (GenBank RefSeq NM\_000698)

(SEQ ID NO: 1)

```

1  gccagggacc agtgggtggga ggaggctgcg gcgctagatg cggacacctg gaccgcccgc
61  ccgaggctcc cggcgctcgc tgctcccgcg gcccgcgcca tgccctcta caccgtcacc
121  gtggccactg gcagccagtg gttcgccggc actgacgact acatctacct cagcctcgtg
181  ggctcggcgg gctgcagcga gaagcacctg ctggacaagc cttctacaa cgacttcgag
241  cgtggcgcgg tggattcata cgacgtgact gtggacgagg aactgggcca gatccagctg
301  gtcagaatcg agaagcgcaa gtactggctg aatgacgact ggtacctgaa gtacatcacg
361  ctgaagacgc cccacgggga ctacatcgag ttcccctgct accgctggat caccggcgat
421  gtcagaggtg tcttgagggg tggacgcgca aagtggccc gagatgacca aattcacatt
481  ctcaagcaac accgacgtaa agaactggaa acacggcaaa aacaatatcg atggatggag
541  tggaaacctg gcttcccctt gagcatcgat gccaaatgcc acaaggattt accccgtgat
601  atccagtttg atagtgaaaa aggagtggac tttgttctga attactcaa agcgatggag
661  aacctgttca tcaaccgctt catgcacatg ttccagtctt cttggaatga cttegccgac
721  tttgagaaaa tctttgtcaa gatcagcaac actatttctg agcgggtcat gaatcactgg
781  caggaagacc tgatgttttg ctaccagttc ctgaatggct gcaacctgtg gttgatccgg
841  cgctgcacag agctgccccg gaagctcccg gtgaccacgg agatggtaga gtgcagcctg
901  gagcggcagc tcagcttggg gcaggaggtc cagcaagggg acattttcat cgtggacttt
961  gagctgctgg atggcatcga tgccaacaaa acagaccctt gcacactcca gttcctggcc
1021  gctcccctct gcttctgtga taagaacctg gccaaacaaga ttgtcccctt tgccatccag
1081  ctcaacaaaa tcccgggaga tgagaacctt attttctct cttcggatgc aaaatacgac
1141  tggcttttgg ccaaaatctg ggtgcgttcc agtgacttcc acgtccacca gaccatcacc
1201  caccttctgc gaacacatct ggtgtctgag gtttttggca ttgcaatgta ccgccagctg

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TABLE 1-continued

Exemplary Sequences	
1261	cctgctgtgc accccatttt caagctgctg gtggcacacg tgagattcac cattgcaatc
1321	aacaccaagg cccgtgagca gctcatctgc gagtgtggcc tctttgacaa ggccaacgcc
1381	acagggggcg gtgggcacgt gcagatggtg cagagggcca tgaaggacct gacctatgcc
1441	tcctgtgct tccccgaggc catcaaggcc cggggcatgg agagcaaaga agacatcccc
1501	tactacttct accgggacga cgggctcctg gtgtgggaag ccatcaggac gttcacggcc
1561	gaggtggtag acatctacta cgagggcgac caggtggtgg aggaggaccg ggagctgcag
1621	gacttcgtga acgatgtcta cgtgtacggc atgcggggcc gcaagtctc aggcttcccc
1681	aagtcggtca agagccggga gcagctgtcg gagtacctga ccgtggtgat cttcacggcc
1741	tcgccccagc acgcccgggt caacttcggc cagtacgact ggtgctcctg gatccccaat
1801	gcgcccccaa ccatcgagc cccgccaccg actgccaaagg gcgtggtgac cattgagcag
1861	atcgtggaca cgctgcccga ccgcccgcgc tctgctggc atctgggtgc agtgtgggcg
1921	ctgagccagt tccaggaaaa cgagctgttc ctgggcatgt acccagaaga gcattttatc
1981	gagaagcctg tgaaggaagc catggcccga ttccgcaaga acctcgaggc cattgtcagc
2041	gtgattgtcg agcgcaacaa gaagaagcag ctgccatatt actactgtc cccagaccgg
2101	attccgaaca gtgtggccat ctgagcacac tgccagtctc actgtgggaa ggccagctgc
2161	cccagccaga tggactccag cctgctcggc aggetgtctg gccaggctc ttggcagtca
2221	catctcttcc tccgaggcca gtacctttcc atttattctt tgatcttcag ggaactgcat
2281	agattgatca aagtgtaaac accatagga cccattctac acagagcagc actgcacagc
2341	gtcctgtcca caccagctc agcatttcca caccaagcag caacagcaaa tcacgaccac
2401	tgatagatgt ctattctgtg tggagacatg ggatgattat tttctgttct atttgtgctt
2461	agtccaatc cttgcacata gtaggtacc aattcaatta ctattgaatg aattaagaat
2521	tggttgccat aaaaataaat cagttcattt aaaaaaaaa aaaaaaa

Human 5-Lipoxygenase Protein Sequence (GenBank RefSeq NM\_000698)

(SEQ ID NO: 2)

MPSYTVTVATGSQWFAGTDDYIYLSLVGSAGCSEKHLLDKPFYNDFERGAVDSYDVTVDEELGEIQLVRIEKRY  
 WLNDWYLKYITLKTTPHGDYIEFPCYRWITGDVEVVLDRGRAKLARDQIHLKQHRRKELETRKQYRWMENP  
 GFPLSIDAKCHKDLPRDIQFDSEKGVDFVLNYSKAMENLFINRFMHMFQSSWNDFADFEKIFVKISNTISERVMN  
 HWQEDLMFGYQFLNGCNPVLIRRCTELPEKLPVTTEMVECSLERQLSLEQEVQGNIFIVDFELLDGIDANKTDP  
 CTLQFLAAPICLLYKNLANKIIVPIAIQLNQIPGDENPIFLPSDAKYDWLLAKIIVRSDDFHVHQTITHLRTHLV  
 SEVFGIAMYRQLPAVHPIFKLLVAHVRFTIAINTKAREQLICEGLFDKANATGGGGHVQMVQRAMKDLTYASLC  
 FPEAIKARGMESKEDIPYYPYRDDGLLVWEAIRFTAEVVDIYYEGDQVVEEDPELQDFVNDVYVYGMGRKSSG  
 FPKSVKSREQLSEYLVVIFTASQAHAAVNFGQYDWCSWIPNAPPTMRAPPPTAKGVVTIEQIVDTLPDRGRSCW  
 HLGAVWALSQFQENELFLGMYPEEHFIEKPVKEAMARFRKNLEAIVSVIAERNKKKQLPYYYLSPDRIPNSVAI

Human FLAP mRNA Sequence (GenBank RefSeq NM\_001629)

(SEQ ID NO: 3)

1 acttcccctt cctgtacagg gcaggttgtg cagctggagg cagagcagtc ctctctgggg  
 61 agcctgaagc aaacatggat caagaaactg taggcaatgt tgtcctggtg gccatcgtca  
 121 ccctcatcag cgtggtccag aatggattct ttgcccataa agtggagcac gaaagcagga  
 181 cccagaatgg gaggagctc cagaggaccg gaacacttgc ctttgagcgg gtctacactg  
 241 ccaaccagaa ctgtgtagat gcgtacccca ctttctctgc tgtgctctgg totgctgggg

TABLE 1-continued

Exemplary Sequences	
301	tactttgcag ccaagttcct gctgcgtttg ctggactgat gtacttgttt gtgaggcaaa
361	agtactttgt cggttacctg ggagagagaa cgcagagcac ccttggttac atatttgga
421	aacgcatcat actcttctctg ttccctcatgt ccggttgctgg catattcaac tattacctca
481	tcttcttttt cggaagtgc tttgaaaact acataaagac gatctccacc accatctccc
541	ctctacttct cattccctaa ctctctgctg aatatggggg tgggtgtctc atctaataca
601	tacctacaag tcatcataat tcagctcttg agagcattct gctcttcttt agatggctgt
661	aaatctattg gccatctggg cttcacagct tgagttaacc ttgcttttcc gggaaacaaa
721	tgatgtcatg tcagctccgc ccctgaaca tgaccgtggc cccaaatttg ctattcccat
781	gcattttgtt tgtttcttca cttatcctgt tctctgaaga tgttttgtga ccaggtttgt
841	gttttcttaa aataaaatgc agagacatgt ttt

Human FLAP Protein Sequence (GenBank RefSeq NM\_001629)

(SEQ ID NO: 4)

MDQETVGNVLLAIVLTLISVVQNGFFAHKVEHESRTQNGRSFQRTGTLAFERVYTANQNCVDAYPTFLAVLWSAG

LLCSQVPAAFAGLMLFVRQKYFVGYLGERTQSTPGYIFGKRIILFLFLMSVAGIFNYLIFFFGSDPENYIKTI

STTISPLLLIP

Human COX-2 mRNA Sequence (GenBank RefSeq NM\_00963)

(SEQ ID NO: 5)

1	caattgtcat acgacttgca gtgagcgtca ggagcacgtc caggaactcc tcagcagcgc
61	ctccttcagc tccacagcca gacgccctca gacagcaaaag cctacccccg cgccgcgccc
121	tgccccccgc tcggatgctc gccccgcgcc tgctgctgtg cgcggctctg gcgctcagcc
181	atacagcaaa tccttgcgtt tcccacccat gtcaaaaccg aggtgtatgt atgagtgtgg
241	gatttgacca gtataagtgc gattgtaccg ggacaggatt ctatggagaa aactgctcaa
301	caccggaatt tttgacaaga ataaaaattat ttctgaaacc cactccaaac acagtgcact
361	acatacttac ccacttcaag ggattttgga acgttgtgaa taacattccc ttctctcgaa
421	atgcaattat gagttatgctc ttgacatcca gatcacattt gattgacagt ccaccaactt
481	acaatgctga ctatggctac aaaagctggg aagccttctc taacctctcc tattatacta
541	gagcccttcc tctctgtcct gatgattgcc cgactccctt ggggtgtcaaa ggtaaaaagc
601	agcttctctga ttcaaatgag attgtgaaa aattgcttct aagaagaaag ttcacccctg
661	atccccaggg ctcaaacatg atgtttgcat tctttgccc gcacttcacg catcagtttt
721	tcaagacaga tcataagcga gggccagctt tcaccaacgg gctgggcccatt ggggtggact
781	taaatcatat ttacggtgaa actctggcta gacagcgtaa actgcgctt ttcaaggatg
841	gaaaaatgaa atatcagata attgatggag agatgtatcc tcccacagtc aaagatactc
901	aggcagagat gatctaccct cctcaagtc ctgagcatct acggtttgcgt gtggggcagg
961	aggctcttgg tctggtgcct ggtctgatga tgtatgccac aatctggctg cgggaacaca
1021	acagagtatg cgatgtgctt aaacaggagc atcctgaatg gggatgatgag cagttgttcc
1081	agacaagcag gctaatactg ataggagaga ctattaagat tgtgatgaa gattatgtgc
1141	aacacttgag tggctatcac ttcaaaactga aatttgacc agaactactt ttcaacaaac
1201	aattccagta ccaaaatcgt attgctgctg aatttaacac cctctatcac tggcatcccc
1261	ttctgcctga cacctttcaa atctatgacc agaaatacaa ctatcaacag tttatctaca
1321	acaactctat attgctggaa catggaatta cccagtttgt tgaatcattc accaggcaaa

TABLE 1-continued

Exemplary Sequences						
1381	ttgctggcag	ggttgctggt	ggtaggaatg	ttccaccgcg	agtacagaaa	gtatcacagg
1441	cttccattga	ccagagcagg	cagatgaaat	accagtcttt	taatgagtac	cgcaaacgct
1501	ttatgctgaa	gccctatgaa	tcatttgaag	aacttacagg	agaaaaggaa	atgtctgcag
1561	agttggaagc	actctatggt	gacatcgatg	ctgtggagct	gtatcctgcc	cttctggtag
1621	aaaagcctcg	gccagatgcc	atctttggtg	aaaccatggt	agaagtggga	gcaccattct
1681	ccttgaagag	acttatgggt	aatgttatat	gttctcctgc	ctactggaag	ccaagcactt
1741	ttggtggaga	agtggggttt	caaatcatca	acactgcctc	aattcagtct	ctcatctgca
1801	ataacgtgaa	gggctgtccc	ttacttcat	tcagtgttcc	agatccagag	ctcattaaaa
1861	cagtcaccat	caatgcaagt	tcttcccgt	ccggactaga	tgatatcaat	cccacagtac
1921	tactaaaaga	acgttcgact	gaactgtaga	agtctaata	tcataattat	ttatttatat
1981	gaaccatgtc	tattaattta	attatttaat	aatatttata	ttaaactcct	tatgttactt
2041	aacatcttct	gtaacagaag	tcagtactcc	tggtgaggag	aaaggagtca	tacttgtgaa
2101	gacttttatg	tcactactct	aaagattttg	ctgttctgt	taagtttggg	aaacagtttt
2161	tattctgttt	tataaaccag	agagaaatga	gttttgacgt	cttttactt	gaatttcaac
2221	ttatattata	agaacgaaag	taaagatggt	tgaatactta	aacactatca	caagatggca
2281	aaatgctgaa	agttttttaca	ctgtcogatg	ttccaatgca	tcttccatga	tgcattagaa
2341	gtaactaatg	ttgaaattt	taaagtactt	ttggttattt	ttctgtcatc	aaacaaaaac
2401	aggatcagc	gcattattaa	atgaatattt	aaattagaca	ttaccagtaa	tttcatgtct
2461	actttttaaa	atcagcaatg	aaacaataat	ttgaaatttc	taaattcata	gggtagaatc
2521	acctgtaaaa	gcttgttttg	tttcttaaag	ttattaaact	tgtacatata	ccaaaaagaa
2581	gctgtcctgg	atttaaatct	gtaaaatcag	atgaaatttt	actacaattg	cttgttaaaa
2641	tattttataa	gtgatgttcc	ttttcacca	agagtataaa	cctttttagt	gtgactgtta
2701	aaacttcctt	ttaaatcaaa	atgccaaat	tattaagggtg	gtggagccac	tgcaagtgtta
2761	tctcaaaaata	agaatatttt	gttgagatat	tccagaattt	gtttatatgg	ctggtaacat
2821	gtaaaatcta	tatcagcaaa	agggctctacc	tttaaaataa	gcaataacaa	agaagaaaac
2881	caaattattg	ttcaaattta	ggtttaaact	tttgaagcaa	actttttttt	atccttgtgc
2941	actgcaggcc	tggtactcag	attttgctat	gaggttaatg	aagtaccaag	ctgtgcttga
3001	ataacgatat	gttttctcag	attttctggt	gtacagttta	atttagcagt	ccatatcaca
3061	ttgcaaaaag	agcaatgacc	tcataaaata	cctcttcaaa	atgcttaaat	tcatttcaca
3121	cattaatttt	atctcagctc	tgaagccaat	tcagtaggtg	cattggaatc	aagcctggct
3181	acctgcatgc	tgttcctttt	cttttcttct	tttagccatt	ttgctaagag	acacagtctt
3241	ctcatcactt	cgtttctcct	attttgtttt	actagtttta	agatcagagt	tcactttctt
3301	tggaactctgc	ctataatttc	ttacctgaac	tttgcaagt	tttcaggtaa	acctcagctc
3361	aggactgcta	tttagctcct	cttaagaaga	ttaaaagaga	aaaaaaaaag	cccttttaaa
3421	aatagtatac	acttatttta	agtgaaaagc	agagaatttt	atttatagct	aattttagct
3481	atctgtaacc	aagatggatg	caaagaggct	agtcctcag	agagaactgt	acggggtttg
3541	tgactggaaa	aagtacggtt	cccattctaa	ttaatgccct	ttcttattta	aaaacaaaac
3601	caaatgatat	ctaagtagtt	ctcagcaata	ataataatga	cgataaact	tctttccac
3661	atctcattgt	cactgacatt	taatggact	gtatattact	taatttattg	aagattatta

TABLE 1-continued

Exemplary Sequences	
3721	tttatgtctt attaggacac tatggttata aactgtgttt aagcctacaa tcattgattt
3781	ttttttgtta tgtcacaatc agtatatddd ctttgggggtt acctctctga atattatgta
3841	aacaatccaa agaaatgatt gtattaagat ttgtgaataa attttttagaa atctgattgg
3901	catattgaga tatttaaggt tgaatgtttg tccttaggat aggcctatgt gctagcccac
3961	aaagaatatt gtctcattag cctgaatgtg ccataagact gaccttttaa aatgttttga
4021	gggatctgtg gatgctctgt taatttgttc agccacaatt tattgagaaa atattctgtg
4081	tcaagcactg tgggttttaa tattttttaa tcaaacgctg attacagata atagtattta
4141	tataaataat tgaaaaaaat tttcttttgg gaagaggggag aaaatgaaat aaatatcatt
4201	aaagataact caggagaatc ttctttacaa ttttactgtt agaatgttta aggttaagaa
4261	agaaatagtc aatagcttg tataaaacac tgttactgtt tttttttaa aaaaaaactt
4321	gatttgttat taacattgat ctgctgacaa aacctgggaa tttgggttgt gtatgcaat
4381	gtttcagtgc ctcagacaaa tgtgtattta acttatgtaa aagataagtc tggaaataaa
4441	tgtctgttta tttttgtact attta

Human COX-2 Protein Sequence (GenBank RefSeq NM\_000963)

(SEQ ID NO: 6)

MLARALLCAVLALSHANPCCSHPCQNRGVCMSVGFQYKDCDTRTGFYGENCSTPEFLTRIKLFLKPTPNTVH  
 YILTHFKGFVNVNIPFLRNAIMSYVLTSRSHLIDSPPTYNADYGYKSWFAFNSLSYTRALPPVDDCPTPLG  
 VKGKKQLPDSNEIVEKLLLRKFIIPDQGSNMMFAFFAQHFTHQFFKTDHKRGPFTNGLGHGVDLNHIYGETLA  
 RQRKRLRFKDKMKYQI IDGEMYPTTKDTQAEMIYPPQVPEHLRFVAVQEVFGLVPLMMYATIWLREHNRVCD  
 VLKQEHPEWDEQLFQTSRLILIGETIKIVIEDYVQHLSGYHFKLKFDELLFNKQFYQNRIAAEFNTLYHWHF  
 LLPDTFQIHDQKYNQQFIYNNSILLEHGI TQFVESFTRQIAGRVAGGRNVPPAVQKVSQAS IDQSRQMKYQSFN  
 EYRKRFLMKPYESFEELTGEKEMSAELEALYGDIDAVELYPALLVEKPRPDAIFGETMVEVGAPPFLKGLMGNVI  
 CSPAYWKPSTFGGEVGFQIINTASIQSLICNNVKGCPFTSFSVPDELIKTVTINASSRSGLDDINPTVLLKER  
 STEL

Human COX-1 mRNA Sequence (GenBank RefSeq NM\_000962)

(SEQ ID NO: 7)

1 aggtgacagc tggaggagg agcgggggtg gagccggggg aagggtgggg aggggatggg  
 61 ctggagctcc gggcagtggt cgaggcgcac gcacaggagc ctgcactctg cgtcccgcac  
 121 cccagcagcc gcgccatgag ccggagtctc ttgctctggt tcttctgtt cctgctcctg  
 181 ctcccgcgcg tcccgtctct gctcgcggac ccaggggcgc ccacgccagt gaatcctctg  
 241 tgttactatc catgccagca ccagggcacg tgtgtccgct tggccttga ccgctaccag  
 301 tgtgactgca cccgcacggg ctattccggc cccaactgca ccatccctgg cctgtggacc  
 361 tggctccgga attcactgag gccacagccc tctttcacc acttcctgct cactcagggg  
 421 cgctggttct gggagtgtgt caatgccacc ttcacccgag agatgctcat gcgcctgta  
 481 ctcacagtgc gctccaacct tatcccagc cccccacct acaactcagc acatgactac  
 541 atcagctggg agtctttctc caactgagc tattacactc gtattctgcc ctctgtgcct  
 601 aaagattgcc ccacacccat gggaaccaa gggaagaagc agttgccaga tgcccagctc  
 661 ctggcccgcg gcttctgct caggaggaag ttcatacctg accccaagg caccaacctc  
 721 atgtttgcct tctttgcaca acacttcacc caccagtctc tcaaaactc tggcaagatg  
 781 ggtcctggct tcaccaagc cttgggcat ggggtagacc tggccacat ttatggagac

TABLE 1-continued

Exemplary Sequences	
841	aatctggagc gtcagtatca actgocggctc ttttaaggatg ggaaactcaa gtaccagggtg
901	ctggatggag aaatgtaccc gccctcggtg gaagaggcgc ctgtgttgat gcaactacccc
961	cgaggcatcc cgccccagag ccagatggct gtgggccagg aggtgttttg gctgcttcct
1021	gggctcatgc tgtatgccac gctctggcta cgtgagcaca accgtgtgtg tgacctgctg
1081	aaggctgagc accccacctg gggcgatgag cagcttttcc agacgacccg cctcatcctc
1141	ataggggaga ccatcaagat tgtcatcgag gagtacgtgc agcagctgag tggctatttc
1201	ctgcagctga aatttgaccc agagctgctg ttcgggtgcc agttccaata ccgcaaccgc
1261	atggccatgg agttcaacca tctctaccac tggcaccccc tcatgcctga ctcttcaag
1321	gtgggctccc aggagtacag ctacgagcag ttcttgttca acacctccat gttggtggac
1381	tatgggggtg aggcctggt ggatgccttc tctcgccaga ttgctggccg gatcgggtggg
1441	ggcaggaaca tggaccacca catcctgcat gtggctgtgg atgtcatcag ggagtctcgg
1501	gagatgcggc tgcagccctt caatgagtac cgcaagaggt ttggcatgaa accctacacc
1561	tccttccagg agctcgtagg agagaaggag atggcagcag agttggagga attgtatgga
1621	gacattgatg cgttgaggatt ctacocctgga ctgcttcttg aaaagtgcc tccaaactct
1681	atctttgggg agagtatgat agagattggg gctccctttt ccctcaaggg tctcctaggg
1741	aatcccatct gttctccgga gtactggaag ccgagcacat ttggcggcga ggtgggcttt
1801	aacattgtca agacggccac actgaagaag ctggtctgcc tcaacaccaa gacctgtccc
1861	tacgtttcct tccgtgtgcc ggatgccagt caggatgatg ggctctgtg ggagcgacca
1921	tccacagagc tctgaggggc aggaaagcag cattctggag gggagagctt tgtgctgtc
1981	attccagagt gctgaggcca gggctgatgg tcttaaatgc tcattttctg gtttggcatg
2041	gtgagtgttg gggttgacat ttagaacttt aagtctcacc cattatctgg aatattgtga
2101	ttctgtttat tcttccagaa tgetgaaact cttgttagcc cttcagattg ttaggagtg
2161	ttctcatttg gtctccaga atactgggtt cttagttgac aacctagaat gtcagatttc
2221	tggttgattt gtaacacagt cattctagga tgtggagcta ctgatgaaat ctgctagaaa
2281	gttagggggt tcttattttg cattccagaa tcttgacttt ctgattgggtg attcaaagtg
2341	ttgtgttctt ggctgatgat ccagaacagt ggctcgtatc ccaaactctg cagcatctgg
2401	ctgtctagaa tgtggatttg attcattttc ctgttcagtg agatatcata gagacggaga
2461	tcctaaggtc caacaagaat gcattccctg aatctgtgcc tgcactgaga gggcaaggaa
2521	gtggggtgtt cttcttggga ccccactaa gacctggctc tgaggatgta gagagaacag
2581	gtgggctgta ttcacgccat tggttggaag ctaccagagc tctatcccca tccaggcttt
2641	gactcatggc agctgtttct catgaagcta ataaaattcg ctttctaaag ttacctgtta
2701	tatatctctt ttggtcccat cctctaaagc agaggcaaca ctggaacatg gctagccttt
2761	cttgtagcca tggctgggctg tgctagaggt tgcagcatga gactttctgc tgggacctt
2821	gggcccata ctgtatagac atgetaccac tggtaacttc tttctcctg cgggccaggc
2881	actgoccttt tcaggaagct ctcttaaat acccattgcc ccagacctgg aagatataac
2941	attcagttcc caccatctga ttaaaacaac ttctccctt acagagcata caacagaggg
3001	ggcaccgggg gaggagagca catactgtgt tccaatttca cgtttttaat tctcatttgt
3061	tctcacacca acagtgtgaa gtgcgtggta taatctccat ttcaaaacca aggaagcagc
3121	ctcagagtgg tcgagtgaca cacctcacgc aggctgagtc cagagettgt gctcctcttg

TABLE 1-continued

Exemplary Sequences	
3181	attcctgggtt tgactcagtt ccaggcctga tcttgectgt ctggctcagg gtcaaagaca
3241	gaatggtgga gtgtagcctc cacctgatat tcaggctact cattcagtc ccaaatatgta
3301	ttttcctaag tgtttactat gtgccagttc ctgtaacagg tgtggggaca cagcagtgag
3361	taatcaatac agacaaggtt ctgcccttat ggagctcaca ctccagtggc agacaaacag
3421	accataaata aggaaacgat gaaataagat atatacaagg tgagtgtgac ttccttcta
3481	acccctctg ctctgtcctc ccctattgcg ctctcaagac cagagacca acagcagtg
3541	tctcagggca gacagccctc cactccagct ctgagaccct tttctcagga cctctgtagg
3601	cagcagagag agaggacaga ggggtaagat gagggttga gggaggttc ttcgatgcc
3661	acactttggg cttagtattt ctgaggaaga gctatggccc agaacaaca ggggaaacta
3721	gagttcggtc tgacagtcct tggggttaag tctcctgtct tatggccag aaactcctgt
3781	ttctccttag ttggctgaa actgctccca tcattccttc tggcctctgc tgaatgcagg
3841	gaatgcaatc cttccctgct cttgcagttg ctctgacgta gaaagatcct tgggtgctg
3901	gaagtctcca tgaagagctt gtgtcctgct ctttcttga gattctattt cccctcttct
3961	gctaatacct cttacttttg ttgagaatcc tctccttct tattaatttc agtcttggtg
4021	gttctatcag ggggtcattc tggccaaggg gtgggcctgt gaatcaatcc tgggcaatca
4081	gacaccctct ccttaaaaaa tggcccgtgg agactgagat cactgactct gactcaatcc
4141	cacagctggc tctgacaaga tggccattt gttcctgctt ccgagatccc cagggcagcc
4201	tggatccctg ccttctcaaa gactttagct tttccttcca tccggtggcc tattccagga
4261	attcctcttt tgcttaaatc agttggagtt tgtgtctggt gcttgtaatc aagcctttat
4321	ggctgctggg ctgagtgaca caagcacttt aatggcctgg agggactttt aatcagtgaa
4381	gatgcaatca gacaagtgtt ttggaaagag caccctcgag aagggtggat gacagggcag
4441	agcaggaagg acaggaagct ggcagaaagg aggaggtgc agccgtggc caaccaggag
4501	ctgatggcag ctggggctag gggagggct ttgaggtgg aaggatggga tgggtccag
4561	aggattcct cctcttaaat caagtgccta gattagtag actttgctta gtattgaca
4621	ctgcacatga aagttttgca aagggaaaca ggctaaatgc accaagaaag cttcttcaga
4681	gtgaagaatc ttaatgctgt taatttaaac atttgttctt ggagttttga tttggtggat
4741	gtgatggttg gttttatttg tcagtttggg tgggctatag cacacagtta tttaatcaaa
4801	cagtaactca ggtgtggctg tgaaggtatt ttgtagatgt gattaacatc tacaatcagt
4861	tgactttaag tgaagagat tacttaata atttgggtga gctgcaactg attagttgaa
4921	aggcctcaag aacaacact gcagtttctt ggaaaagaag aaactttgcc tcaagactat
4981	agccatcgac tctgctga gttccagcc tgctagtctg ccctatggat ttgaagtttg
5041	ccaaccccaa caattgtgtg aattaatttc taaaataaa gctatataca gcc

Human COX-1 Protein Sequence (GenBank RefSeq NM\_000962)

(SEQ ID NO: 8)

MSRSLLLWFLFLFLLLPPLVLLADPGAPT FVNPCYYPCQHQGI CVRFGLDRYQCDCRTRTGYSGPNCTIPGLWT  
 WLRNSLRPSPSFTHFLLTGHRWFWEFVNATFIREMLMRLVLTVRSNLIPSPPTYNSAHDYISWESFSNVSYTRI  
 LPSVPKDCPTPMGTLKQKQLPDAQLLARRFLRRKFI PDPQGTNLMFAFQAQHFTHQFFKTS GKMPGPFKALGH  
 GVDLGHYIGDNLERQYQLRFLKDKLKYQVLDGEMYPSPVEEAPVLMHYPRGIPQSQMAVQEVFGLPLMLY  
 ATLWLREHNRVCDLLKAEHPTWGDEQLFQTRRLILIGETIKIVIEEYVQQLSGYFLQLKFDPELLFGVQFYRNR  
 IAMEFNHLYHWHPLMPDSFKVGSQEYSYEQFLFNNTSMLVDYGVLEALVDAFSRQIAGRIGGGRNMDHHILHVAVDV

TABLE 1-continued

Exemplary Sequences

IRESREMLQLPQFNEYRKRFRGKMPYTSFQELVGEKEMAAELEEYGDIDALEFYPLGKLLLEKCHPNSIFGSMIEIG

APFSLKGLLGNPICSPYWKPFSTFGGEVGFNIVKTATLKKLVCLNTKTCYPVSRVDPASQDDGPAVERPSTEL

<sup>1</sup>Similarity between mRNA sequences.

<sup>2</sup>Similarity between protein sequences.

## II. 5-LIPOXYGENASE INHIBITORS

The applicant has discovered that inhibiting the activity of 5-lipoxygenase promotes osteogenesis which can be used to accelerate and/or enhance the healing of a bone fracture, to treat a bone defect, or to treat by inducing bone formation. The applicant's discovery is based on his hypothesis that a potential mechanism by which loss of COX-2 function could inhibit fracture healing was by shunting arachidonic acid into the lipoxygenase pathway with consequent formation of abnormally high inhibitory 5-HETE, LTB<sub>4</sub>, or other 5-LO metabolite levels (FIG. 2). During a normal inflammation response, such as a fracture, the synthesis of prostaglandins and leukotrienes is balanced (FIG. 2A). Without being bound to a theory, the inventor theorizes that inhibiting COX-2 function shunts arachidonic acid into the lipoxygenase pathway to produce excess leukotrienes thereby impairing bone formation (FIG. 2B). Conversely, by inhibiting 5-lipoxygenase activity, arachidonic acid is shunted into the cyclooxygenase pathway to produce excess prostaglandins that accelerate or enhance bone formation (FIG. 2C).

To test this potential mechanism, fracture healing was assessed in 5-LO<sup>-/-</sup> mice. The applicant found that loss of 5-LO function accelerates healing. Radiographic examination of fracture healing in age-matched mice in the C57BL/6 background showed that fracture bridging occurred by 2 weeks post-fracture in the 5-LO<sup>-/-</sup> mice as compared to 3 weeks post-fracture in the normal mice (FIG. 3). Further, callus remodeling was significantly accelerated, thus the 5-LO<sup>-/-</sup> callus regains its initial structural and material properties much faster than in normal mice based upon torsional mechanical testing (FIG. 4 and TABLE 2). Thus, loss of 5-LO function accelerates and enhances fracture healing and bone formation.

Histological examination of calcified samples supported the radiographic data. Plastic embedded, calcified sections of normal and 5-LO<sup>-/-</sup> mouse fractures stained with Stevenel's blue and van Gieson's picrofuchsin show that after just 2 weeks of healing the fracture was bridged with calcified tissue in the 5-LO<sup>-/-</sup> mice while the normal mouse (C57BL/6) still had a cartilaginous soft callus. Histomorphometric measurements of fracture callus cartilage area showed that cartilage area peaked by day 7 post-fracture in 5-LO<sup>-/-</sup> mice and by day 10 post-fracture in normal mice (FIG. 5 and TABLE 3). Measurement of new bone (calcified tissue) in the fracture callus showed that almost twice as much new bone in the 5-LO<sup>-/-</sup> after 7 days of healing and significantly more new bone at day 10 as well (FIG. 5 and TABLE 2). These data show that a normal, albeit significantly accelerated, endochondral ossification pathway is used to heal the fracture in the 5-LO<sup>-/-</sup> mice. Experiments using younger and older 5-LO<sup>-/-</sup> mice and in different genetic backgrounds gave identical results: loss of 5-LO function results in accelerated bone regeneration.

The data from these experiments show that a 10 day fracture callus in 5-LO<sup>-/-</sup> mouse is equivalent to a 14 day callus

in a normal mouse; that a 14 day 5-LO<sup>-/-</sup> callus is equivalent to a 21 day normal callus; and that a 1 month 5-LO<sup>-/-</sup> callus is equivalent to a 3 month normal callus (FIG. 3). Thus, loss of 5-LO function accelerates and/or enhances the regenerative and remodeling phases of fracture healing.

In one aspect of the invention, compounds that inhibit 5-lipoxygenase activity accelerate and/or enhance healing of a bone fracture or prevent bone resorption or promote bone formation provide important benefits to efforts at treating human disease. Compounds that inhibit 5-lipoxygenase activity can be used, e.g., in a method for treating bone fracture due to trauma, or due to osteoporosis or osteoarthritis, in a method for treating Paget's disease, in a method for treating other conditions such as bone transplants and diseases associated with increased bone fracture, and in methods that require bone formation such as spine fusions, other bone and joint ankylosis procedures, bone or limb lengthening, augmentation of bone structure, incorporation of allograft, autograft, or synthetic bone material into bone defects, bone growth into or around prosthetic devices, and other similar procedures.

Several inhibitors of 5-lipoxygenase and their dosing are known which are useful for practicing the methods of the invention. A 5-lipoxygenase inhibitor can be 3-[1-(4-chlorobenzyl)-3-t-butyl-thio-5-isopropylindol-2-yl]-2,2-dimethylpropanoic acid (MK886) or derivatives thereof; 3-(1-(4-chlorobenzyl)-3-(1-butyl-thio)-5-(quinolin-2-yl-methoxy)-indol-2-yl)-2,2-dimethyl propanoic acid (MK-591) or derivatives thereof; nordihydroguaiaretic acid (NDGA) or derivatives thereof; 2-(12-hydroxydodeca-5,10-dienyl)-3,5,6-trimethyl-1,4-benzoquinone (AA861) or derivatives thereof; or (N-(1-benzo(b)thien-2-ylethyl)-N-hydroxyurea) (Zileuton) or derivatives thereof. Derivatives include, e.g., pharmaceutically acceptable salts, prodrugs, etc. which also are useful as 5-lipoxygenase inhibitors. Derivatives of exemplary compounds are intended to be within the scope of the claimed invention.

Other 5-lipoxygenase inhibitors for use in the invention include masoprocol, tenidap, flobufen, lonapalene, tagorizine, Abbott A-121798, Abbott A-76745, Abbott A-78773, Abbott A-79175, Abbott ABT 761, Dainippon AL-3264, Bayer Bay-x-1005, Biofor BF-389, bunaprolast, Cytomed CMI-392, Takeda CV-6504, enazadrem phosphate, Leo Denmark ETH-615, flezelastine hydrochloride, Merck Frosst L-663536, Merckle ML-3000, 3M Pharmaceuticals R-840, rilopirox, Schering Plough SCH-40120, tepoxalin, linazolast (TMK-688), Zeneca ZD-2138, Bristol-Myers Squibb BU-4601A, carbazomycin C, lagunamycin, Wellcome BW-70C, Ciba-Geigy CGS-26529, Warner-Lambert CI 1004, Warner-Lambert PD-136005, Warner-Lambert PD-145246, Elsai E-3040, Fujirebio F-1322, Fujisawa FR-110302, Merck Frosst L-699333, Merck Frosst L-739010, Lilly LY-269415, Lilly LY-178002, Hoechst Roussel P-8892, SmithKline Beecham SB-202235, American Home Products WAY-121520, American Home Products WAY-125007, Zeneca ZD-7717, Zeneca ZM-216800, Zen-

eca ZM-230487, 1,2-dihydro-n-(2-thiazolyl)-1-oxopyrrolo (3,2,1-kl)phenothiazine-1-carboxamide, Abbott A-65260, Abbott A-69412, Abbott-63162, American Home Products AHR-5333, Bayer Bay-q-1531, Boehringer Ingelheim BI-L-357, Boehringer Ingelheim BI-L-93BS, Boehringer Ingelheim BIL 226XX, Bristol-Myers Squibb BMY-30094, carbazomycin B, Wellcome BW-B218C, Chauvin CBS-1114, Ciba-Geigy CGS-21595, Ciba-Geigy CGS-22745, Ciba-Geigy CGS-23885, Ciba-Geigy CGS 24891, Ciba-Geigy CGS-8515, Chiesi CHF-1909, Warner-Lambert CI-986, Warner-Lambert CI-987, cirsiliol, docebenone, Eisai E-5110, Eisai E-6080, enofelast, eprocabazolin-A, eprovaferen, evandamine, Fisons FPL 62064, Zeneca ICI-211965, Zeneca ICI-216800, Kyowa Hakko KF-8940, Merck & Co L-651392, Merck & Co L-651896, Merck & Co L-652343, Merck & Co L-656224, Merck & Co L-670630, Merck & Co L-674636, Lilly LY-233569, Merck & Co MK-591, Merck & Co L-655240, nitrosoxacin-A, Ono ONO-5349, Ono ONO-LP-219, Ono ONO-LP-269, Warner-Lambert PD-127443, Purdue Frederick PF-5901, Rhone-Poulenc Rorer Rev-5367, Rhone-Poulenc Rorer RG-5901-A, Rhone-Poulenc Rorer RG-6866, Roussel-Uclaf RU-46057, Searle SC-41661A, Searle SC-45662, Sandoz SDZ-210-610, SmithKline Beecham SK&F-104351, SmithKline Beecham SK&F-104493, SmithKline Beecham SK&F-105809, Synthelabo SL-81-0433, Teijin TEI-8005, Terumo TMK-777, Terumo TMK-781, Terumo TMK-789, Terumo TMK-919, Terumo TMK-992, Teikoku Hormone TZI-41127, American Home Products WAY-120739, American Home Products WY-47288, American Home Products WY-48252, American Home Products WY-50295, Yoshitomi Y-19432, 4-{3-[4-(2-methyl-1H-imidazol-1-yl)phenylthio]}-phenyl-3,4,5,6-tetrahydro-2H-pyran-4-carboxamide, esculetin, phenidone and its derivatives, BI-L-239, 5,8,11-eicosatriynoic acid (ETI), 5,8,11,14-eicosatetraynoic acid (ETYA), cinnamyl-3,4-dihydroxy-alpha-cyanocinnamate, curcumin, esculetin, gossypol, caffeic acid, baicalein, 7,7-dimethyleicosadrenoic acid (DEDA), Ly311727, bromoenol lactone, methyl arachidonyl fluorophosphonate, methyl  $\gamma$ -linolenyl fluorophosphonate, oleyoxyethyl phosphorylcholine, AACOCF<sub>3</sub>, n-(p-amylnamoyl) anthranilic acid, mepacrine, quinacrine, atabrine, parabromophenacylbromide, aristolochic acid, corticosteroids, Glaxo SmithKline 480848, Glaxo SmithKline 659032, Glaxo SmithKline 677116, BMS-181162, MJ33, and Millennium Pharmaceuticals MLN977.

More preferred 5-lipoxygenase inhibitors include masoprocol, tenidap, zileuton, flobufen, lonapalene, tagorizine, Abbott A-121798, Abbott A-76745, Abbott A-78773, [(R)(+) N'-[[5-(4-fluorophenoxy)furan-2-yl]-1-methyl-2-propynyl]-N-hydroxyurea (Abbott A-79175),] Abbott A-79175, Abbott ABT 761, Dainippon AL-3264, Bayer Bay-x-1005, Biofor BF-389, bunaprolast, Cytomed CMI-392, Takeda CV-6504, Ciba-Geigy CGS-26529, enazadrem phosphate, Leo Denmark ETH-615, flezelastine hydrochloride, Merck Frosst L 663536, Merck Frosst L 699333, Merckle ML-3000, 3M Pharmaceuticals R-840, rilopirox, Schering Plough SCH 40120, tepoxalin, linazolast (TMK-688), Zeneca ZD-7717, Zeneca ZM-216800, Zeneca ZM-230487, Zeneca ZD-2138; and NDGA (nondihydroguaiaretic acid).

Even more preferred 5-lipoxygenase inhibitors include tenidap, zileuton, flobufen, lonapalene, tagorizine, AA-861, Abbott A-121798, Abbott A-76745, Abbott A-78773, Abbott A-79175, Abbott ABT 761, Ciba-Geigy CGS-26529, Biofor BF-389, Cytomed CMI-392, Leo Denmark ETH-615, lonapalene, Merck Frosst L 699333, Merckle ML-3000, 3M Pharmaceuticals R-840, linazolast (TMK-688), Zeneca ZD-7717,

Zeneca ZM-216800, Zeneca ZM-230487, Zeneca ZD-2138, and NDGA (nondihydroguaiaretic acid).

In another aspect, the invention comprises a 5-LO inhibitor and a COX inhibitor and its use. Preferably, the COX inhibitor is a selective COX-1 inhibitor, i.e., that it inhibits the activity of COX-1 more than it inhibits the activity of COX-2. The use of a 5-LO inhibitor and a COX inhibitor is intended to embrace administration of each inhibitor in a sequential manner in a regimen that will provide beneficial effects of the drug combination, the co-administration of the inhibitors in a substantially simultaneous manner, such as in a single capsule having a fixed ratio of these active agents, or in multiple, separate capsules for each agent, as well as a single compound that inhibits both enzymes.

The COX inhibitor can be selected from the group consisting of celecoxib; rofecoxib; meloxicam; piroxicam; valdecoxib, parecoxib, etoricoxib, CS-502, JTE-522; L-745,337; FR122047; NS398; from non-selective NSAIDs that would include aspirin, ibuprofen, indomethacin CAY10404, diclofenac, ketoprofen, naproxen, ketorolac, phenylbutazone, tolfenamic acid, sulindac, and others, or from steroids or corticosteroids. Compounds which selectively inhibit cyclooxygenase-2 have been described in U.S. Pat. Nos. 5,380,738, 5,344,991, 5,393,790, 5,466,823, 5,434,178, 5,474,995, 5,510,368 and WO documents WO96/06840, WO96/03388, WO96/03387, WO95/15316, WO94/15932, WO94/27980, WO95/00501, WO94/13635, WO94/20480, and WO94/26731, and are otherwise known to those of skill in the art.

Selective COX-1 inhibitors are known in the art. The following is a list of preferred COX-1 selective NSAIDs: SC-560 [Smith et al., Proceedings of the National Academy of Sciences of the United States of America 95:13313-8 (1998)], FR122047 [Dohi et al., European Journal of Pharmacology 243:179-84 (1993)], Valeroyl salicylate, Aspirin. Aspirin is an irreversible cyclooxygenase inhibitor that is rapidly inactivated in vivo. While aspirin can inhibit COX-1 and COX-2, prior treatment with aspirin can inactivate all pre-existing COX-1 before or during expression of COX-2. Thus any new COX-2 that is expressed is active but all "older" COX-1 or COX-2 is inactivated.

The following is a list of NSAIDs that preferentially inhibit COX-1 versus COX-2: Dexketoprofene, Keterolac, Flurbiprofen, Suprofen. See also [Warner et al., Proceedings of the National Academy of Sciences of the United States of America 96:7563-8 (1999)].

In another embodiment, the invention comprises a 5-LO inhibitor and a COX-2 activator and its use. COX-2 activators also are known in the art. See [Tanabe and Tohna, Prostaglandins & other Lipid Mediators 68-69:95-114 (2002)] for review article concerning regulation of COX-2 gene expression and as a reference for those compounds or treatments listed below without a reference. Preferred COX-2 activators include ultrasound therapy [Sena et al., Ultrasound in Medicine & Biology 31:703-8 (2005)], pulsed electromagnetic fields (PEMF) [Lohmann et al., Journal of Orthopaedic Research 21:326-34 (2003)], BMP2 [Chikazu et al., Journal of Bone and Mineral Research 17:1430-40 (2002)], PDGF, FGF, and PTH and its analogs (PTHrP and teraparotide) [Maciel et al., Journal of Rheumatology 24:2429-35 (1997)]. Other COX-2 activators include Prostaglandins and prostaglandin receptor agonists [Rosch et al., Biochemical and Biophysical Research Communications 338:1171-8 (2005)], PDGF (platelet derived growth factor), IL-1alpha (interleukin 1 alpha), IL-1beta, TNF-alpha (tumor necrosis factor alpha), FGF (fibroblast growth factor), TGF-beta (transform-

ing growth factor beta), TGF-alpha, EGF (epidermal growth factor), TPA (tetradecanoyl phorbol acetate),

In addition, the invention comprises a combination comprising a therapeutically-effective amount of a 5-lipoxygenase inhibitor and a cyclooxygenase-2 inhibitor, such as, e.g., licochalcone, Dupont Dup 697, Taisho NS-398, meloxicam, flosulide, Glaxo SmithKline 406381, Glaxo SmithKline 644784, or tepoxalin.

The modulation of bone metabolism by the methods of the invention can be determined by examination of bone strength and mass after administration compared to a control subject. Such examination can be performed in situ by using imaging techniques (e.g., X-ray, nuclear magnetic resonance imaging, X-ray tomography, ultrasound, and sound conduction) or stress testing, or ex vivo by standard histological, radiographic, mechanical, or biochemical methods. Modulation of bone density and/or bone mass can be assessed by changes in one or more parameters such as bone mineral density, bone strength, trabecular number, bone size, and bone tissue connectivity. Several methods for determining bone mineral density (BMD) are known in the art. For example, BMD measurements may be done using, e.g., dual energy xray absorptiometry or quantitative computed tomography, and the like. Similarly, increased bone formation can be determined using methods well known in the art. For example, dynamic measurements of bone formation rate (BFR) can be performed on tetracycline labeled cancellous bone from the lumbar spine and distal femur metaphysis using quantitative digitized morphometry (Ling et al., *Endocrinology* 140: 5780-5788 (1999)). Alternatively, bone formation markers, such as alkaline phosphatase activity, serum collagen peptide levels, or serum osteocalcin levels can be assessed to indirectly determine whether increased bone formation has occurred (Looker et al., *Osteoporosis International* 11: 467-480 (2000)). Compounds that modulate an arachidonic acid metabolic or signaling pathway can be tested for their ability to accelerate or enhance fracture healing and/or bone formation, promote bone formation, and prevent bone loss. This can be tested in a variety of animal models well known to one skilled in the art such as animal fracture models, animal osteotomy models, animal skull trephine defect models, animal bone defect models, various animals segmental defect models and bone lengthening models, ovariectomy induced bone loss models, and the like. The utility of these animal models is well established and is supported by a wide range of different observations. For example, BMP2 studies in animals including rats demonstrated that BMP2 stimulates osteogenesis and BMP2 is now used clinically in humans for bone repair applications (tradename INFUSE). There are hundreds of papers about this in animals and tens of papers about humans; NSAIDs inhibit fracture repair in rats [Simon et al., *Cyclooxygenase 2 function is essential for bone fracture healing. Journal of Bone and Mineral Research* 17:963-76 (2002)] and NSAID use has been correlated to poor fracture healing in humans [Burd et al., *Journal of Bone and Joint Surgery (British)* 85B:700-5 (2003)]; studies cited in Rubin et al. (2001), *JBJS* 83(2):259-270 indicating that ultrasound treatment accelerates fracture repair in rats (Azuma ref.) and in humans. FDA guidelines for osteoporosis therapies indicate that preclinical studies require use of 2 species and that one must be an ovariectomized rat model.

Modulation of bone metabolism by the methods of the invention can be determined in vitro by examining the proliferation, survival, and differentiation of osteoblasts and/or chondrocytes following treatment that alters arachidonic acid metabolism as compared to mock treated cells. Treatment of cells or organ explants such as newborn rodent calvaria or

phalanges can be with compounds that inhibit 5-lipoxygenase activity, alter cyclooxygenase activity, affect leukotriene or prostaglandin receptor function, and the like as set forth in this application. Additional treatment methods can include use of antisense nucleic acids, interfering RNAs, other nucleic acid or proteins, and the like. Osteoblast or chondrocyte proliferation and survival can be measured by a number of techniques well known to one skilled in the arts such as cell counting, incorporation of radiolabeled thymidine or bromodeoxyuridine into replicating DNA, trypan blue exclusion, and terminal deoxynucleotidyl transferase end labeling of DNA within cells undergoing apoptosis. Differentiation of osteoblasts and/or chondrocytes can be measured by a number of techniques well known to one skilled in the arts and would include formation of mineralized nodules stained by the method of von Kossa or with alizarin red to ascertain osteoblast or chondrocyte culture mineralization, alcian blue staining of chondrocytes to measure elaboration of proteoglycan matrix, gene expression analyses to measure markers of osteoblast and chondrocyte differentiation such as Type I, Type II, and Type X collagen, osteocalcin, and aggrecan using protein or nucleic acid based assay methods, measurement of alkaline phosphatase activity, and measurement of RANKL, OPG, VEGF, bone morphogenetic protein, and other growth factors by quantitative methods such as enzyme-linked immuno assays (EIA).

#### 5-Lipoxygenase-Activating Protein (FLAP)

FLAP is an 18-kD membrane-bound polypeptide which specifically binds arachidonic acid and activates 5-LO by acting as an arachidonic acid transfer protein. The FLAP gene spans greater than 31 kb and consists of five small exons and four large exons (GenBank 182657, Genbank M60470 for exon 1, Genbank M63259 for exon 2, Genbank M63260 for exon 3, Genbank M63261 for exon 4, and Genbank M63222 for exon 5).

The nuclear envelope is the intracellular site at which 5-LO and FLAP act to metabolize arachidonic acid, and ionophore activation of neutrophils and monocytes results in the translocation of 5-LO from a nonsedimentable location to the nuclear envelope. Inhibitors of FLAP function prevent translocation of 5-LO from cytosol to the membrane and inhibit 5-LO activation. Thus, FLAP inhibitors are anti-inflammatory drug candidates.

Leukotriene synthesis is reduced by drugs that inhibit FLAP (MK866) or in mice lacking FLAP. Thus, in one aspect of the invention, FLAP inhibitors such as BAYx 1005, MK-886, and MK-0591, are used in methods that modulate an arachidonic acid metabolic or signaling pathway thereby accelerating and/or enhancing fracture healing and bone formation.

#### Antisense Treatment

The term "antisense nucleic acid" is intended to refer to an oligonucleotide complementary to the base sequences of 5-LO or FLAP-encoding DNA and RNA or those that encode other proteins in an arachidonic acid metabolic or signaling pathway. Antisense oligonucleotides can be modified or unmodified RNA, DNA, or mixed polymer oligonucleotides, and, when introduced into a target cell, specifically bind to their target nucleic acid and interfere with transcription, RNA processing, transport and/or translation. Targeting double-stranded (ds) DNA with oligonucleotide leads to triple-helix formation; targeting RNA will lead to double-helix formation.

Antisense constructs can be designed to bind to the promoter and other control regions, exons, introns or even exon-intron boundaries of a gene. Antisense RNA constructs, or DNA encoding such antisense RNAs, can be employed to

inhibit gene transcription or translation or both within a host cell, either in vitro or in vivo, such as within a host animal, including a human subject. Nucleic acid sequences comprising “complementary nucleotides” are those which are capable of base-pairing according to the standard Watson-Crick complementarity rules, where guanine pairs with cytosine (G:C) and adenine pairs with either thymine (A:T) in the case of DNA, or adenine pairs with uracil (A:U) in the case of RNA.

While all or part of the gene sequence may be employed in the context of antisense construction, preferably any sequence 17 bases long can be used to specify a unique target sequence. Although shorter oligomers are easier to make and increase in vivo accessibility, numerous other factors are involved in determining the specificity of hybridization. The antisense oligonucleotide is selected such that the binding affinity and sequence specificity to its complementary target is sufficient for use as therapeutic agents. Thus, oligonucleotides of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22 or more base pairs can be used. One can readily determine whether a given antisense nucleic acid is effective at targeting of the corresponding host cell gene simply by testing the constructs in vitro to determine whether the endogenous gene's function is affected or whether the expression of related genes having complementary sequences is affected.

#### Interfering RNA

Interfering RNA (RNAi) fragments, particularly double-stranded (ds) RNAi, can be used to modulate an arachidonic acid metabolism or signaling pathway. Small interfering RNA (siRNA) are typically 19-25 nucleotide-long RNA molecules that interfere with the expression of genes. Methods relating to the use of RNAi to silence genes in *C. elegans*, *Drosophila*, plants, and humans are known in the art (Fire et al., Nature 391: 806-811 (1998); Sharp, P. A. RNA interference 2001. Genes Dev. 15: 485-490 (2001); Tuschl, T. Chem. Biochem. 2: 239-245 (2001); WO0129058; and WO9932619).

The nucleotide sequence employed RNAi comprises sequences that are at least about 15 to 50 basepairs. The sequence can be a duplex, optionally with overhangs at the 5'-end and/or the 3'-end, where one strand of the duplex comprises a nucleic acid sequence of at least 15 contiguous bases having a nucleic acid sequence of a nucleic acid molecule within an arachidonic acid metabolic or signaling pathway. The length of each strand can be longer where desired, such as 19, 20, 21, 22, 23, 24, 25, or 30 nucleotides or up to the full length of any of those described herein. The single-stranded overhang can be, for example, 1, 2, 3, 4, 5, or 10 nucleotides long, and can be present at the 3'-end, the 5' end, or both the 3'-end and the 5'-end. Such fragments can be readily prepared by directly synthesizing the fragment by chemical synthesis, by application of nucleic acid amplification technology, or by introducing selected sequences into recombinant vectors for recombinant production.

In particular, the nucleotide sequences or RNAi can be oligonucleotides complementary to the base sequences of 5-LO or FLAP-encoding DNA and RNA or to the base sequences encoding other proteins in an arachidonic acid metabolism or signaling pathway. The oligonucleotides can be modified or unmodified RNA, DNA, or mixed polymer oligonucleotides, and, when introduced into a target cell, specifically bind to their target nucleic acid and interfere with transcription, RNA processing, transport and/or translation.

#### Other Agents

In another aspect of the invention, an additional agent or drug may be administered to the subject. The additional agent can contain one or more active agents that effectively regulate

calcium homeostasis, modulate chondrogenesis, modulate osteogenesis, modulate bone remodeling, regulate pain, regulate inflammation, or have antibiotic activity. The additional active agent can be, but is not limited to, an estrogen, an IGF, insulin, bone morphogenetic proteins and other growth factors, osteoprotegerin (OPG), a calcitonin, a bisphosphonate, vitamin D<sub>3</sub> or an analogue thereof, a statin, an adrogen, a fluoride salt, a parathyroid hormone or an analogue thereof, agents that enhance angiogenesis such as vascular endothelial growth factor (VEGF), agents that alter regulation of transcription of naturally occurring hormone regulators involved in bone metabolism, a vitamin, a mineral supplement, a nutritional supplement, and combinations thereof. The additional agent also may be an antibiotic such as gentamycin, ciprofloxacin, vancomycin, and/or others. This additional active agent can be administered to the subject prior to, concurrently with or subsequently to administration of the 5-lipoxygenase inhibitor of this invention. Anti-inflammatory drugs, including but not limited to nonsteroidal anti-inflammatory drugs and corticosteroids, p38 kinase inhibitors, and antiviral drugs, including but not limited to ribivirin, vidarabine, acyclovir and ganciclovir, may also be combined in compositions of the invention. Antibiotic compounds including but not limited to gentamicin, teicoplanin, tobramycin, and vancomycin, may also be combined in the composition of the invention.

### III. PHARMACEUTICAL FORMULATIONS AND MODES OF ADMINISTRATION

The methods described herein use pharmaceutical compositions comprising the molecules described above, together with one or more pharmaceutically acceptable excipients or vehicles, and optionally other therapeutic and/or prophylactic ingredients. Such excipients include liquids such as water, saline, glycerol, polyethyleneglycol, hyaluronic acid, ethanol, cyclodextrins, modified cyclodextrins (i.e., sulfobutyl ether cyclodextrins) etc. Suitable excipients for non-liquid formulations are also known to those of skill in the art. Pharmaceutically acceptable salts can be used in the compositions of the present invention and include, for example, mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like. A thorough discussion of pharmaceutically acceptable excipients and salts is available in *Remington's Pharmaceutical Sciences*, 18th Edition (Easton, Pa.: Mack Publishing Company, 1990).

Additionally, auxiliary substances, such as wetting or emulsifying agents, biological buffering substances, surfactants, and the like, may be present in such vehicles. A biological buffer can be virtually any solution which is pharmacologically acceptable and which provides the formulation with the desired pH, i.e., a pH in the physiologically acceptable range. Examples of buffer solutions include saline, phosphate buffered saline, Tris buffered saline, Hank's buffered saline, and the like.

Depending on the intended mode of administration, the pharmaceutical compositions may be in the form of solid, semi-solid or liquid dosage forms, such as, for example, tablets, suppositories, pills, capsules, powders, liquids, suspensions, creams, ointments, lotions or the like, preferably in unit dosage form suitable for single administration of a precise dosage. The compositions will include an effective amount of the selected drug in combination with a pharmaceutically acceptable carrier and, in addition, may include other pharmaceutical agents, adjuvants, diluents, buffers, etc.

The invention includes a pharmaceutical composition comprising a compound of the present invention including isomers, racemic or non-racemic mixtures of isomers, or pharmaceutically acceptable salts or solvates thereof together with one or more pharmaceutically acceptable carriers, and optionally other therapeutic and/or prophylactic ingredients.

In general, compounds of this invention will be administered as pharmaceutical formulations including those suitable for oral (including buccal and sub-lingual), rectal, nasal, topical, pulmonary, vaginal or parenteral (including intramuscular, intraarterial, intrathecal, subcutaneous and intravenous) administration, in a form suitable for administration by inhalation or insufflation, or in a form suitable for administration at the bone formation site. The preferred manner of administration is oral or intravenous using a convenient daily dosage regimen which can be adjusted according to the degree of affliction.

Formulations for delivery at the bone formation site include adsorption onto or encapsulation within polylactide and/or polygalactide polymers, palmitic acid, alginate, plaster, calcium sulfate, calcium phosphate, mixtures of calcium sulfate and calcium phosphate, hydroxyapatite, collagen or other extracellular matrix material, bone wax (such as that from CP Medical, Inc., Ethicon, Inc., Unites States Surgical Corp., or Ceremed), Orthocon Bone Putty (a mixture of calcium stearate, vitamin E acetate, and alkylene oxide copolymer) or other materials or compounds that can be used for this purpose. Delivery can be accomplished by direct placement at the bone formation site or by deposition of the active compound of the invention with or without a carrier onto the surface of prosthetic or surgically implanted devices.

A pharmaceutically or therapeutically effective amount of the composition is delivered to the subject. The precise effective amount varies from subject to subject and depends upon the species, age, the subject's size and health, the nature and extent of the condition being treated, recommendations of the treating physician, and the therapeutics or combination of therapeutics selected for administration. Thus, the effective amount for a given situation can be determined by routine experimentation. For purposes of the present invention, generally a therapeutic amount will be in the range of about 0.05 mg/kg to about 40 mg/kg body weight, more preferably about 0.5 mg/kg to about 20 mg/kg, in at least one dose. In larger mammals the indicated daily dosage can be from about 1 mg to 4,800 mg, one or more times per day, more preferably in the range of about 10 mg to 1,200 mg. The subject may be administered as many doses as is required to reduce and/or alleviate the signs, symptoms, or causes of the disorder in question, or bring about any other desired alteration of a biological system. One of ordinary skill in the art of treating such diseases will be able, without undue experimentation and in reliance upon personal knowledge and the disclosure of this application, to ascertain a therapeutically effective amount of the compounds of this invention for a given disease. When practicing the methods of the invention starting human doses may need to be estimated from rat dose data. Such estimation methods are well known in the art. See FDA publication "Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers" published July 2005 (Federal Register Document 5-14456) and available online at

[www.fda.gov/Cder/guidance/5541fnl.pdf](http://www.fda.gov/Cder/guidance/5541fnl.pdf). In general, the rat dose expressed as mg/kg should be divided by 6.2 to obtain an equivalent human dose.

When desired, formulations can be prepared with enteric coatings adapted for sustained or controlled release administration of the active ingredient.

The pharmaceutical preparations are preferably in unit dosage forms. In such form, the preparation is subdivided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged preparation, the package containing discrete quantities of preparation, such as packeted tablets, capsules, and powders in vials or ampoules. Also, the unit dosage form can be a capsule, tablet, cachet, or lozenge itself, or it can be the appropriate number of any of these in packaged form.

#### IV. EXPERIMENTAL

Below are examples of specific embodiments for carrying out the present invention. The examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperatures, etc.), but some experimental error and deviation should, of course, be allowed for.

##### Example 1

##### 5-LO Knock Out Mice

Knock out mice lacking 5-lipoxygenase (Alox5<sup>-/-</sup> or 5-LO<sup>-/-</sup>) were purchased from Jackson Laboratory, Bar Harbor, Me. An impending femur fracture was stabilized with an intramedullary wire that was inserted retrograde into the femoral canal. A three-point bending device was used to make the fracture. Femur fracture healing was measured or assessed by histomorphometry, radiography, and torsional mechanic testing. The 5-LO<sup>-/-</sup> mice demonstrated statistically significant, quantitative acceleration and enhancement of fracture healing as compared to wild-type mice of identical genetic background and age (C57BL/6). Closed mid-diaphyseal fractures were made in 10-12 week old female mice. Fracture healing was assessed by x-rays (FIG. 3) and quantitatively assessed by torsional mechanical testing 4 and 12 weeks after fracture (FIG. 4 and TABLE 2). After 4 or 12 weeks of healing, the fractured femurs from 5LO<sup>-/-</sup> and wild type (WT) mice were excised and mechanically tested to failure in torsion using an MTS servohydraulic test machine and Interface 20 Nm torque load cell. Fractured femur dimensions were measured before and after testing. Peak torque, rigidity, maximum shear stress, and shear modulus were calculated from callus dimensions and the torque to angular displacement curves. All mechanical parameters were 50-120% higher after 4 weeks of healing in the 5-LO<sup>-/-</sup> as compared to the WT mice. Histomorphometric analysis of time-staged fracture specimens from normal and 5-LO<sup>-/-</sup> mice showed that cartilage area peaked early and to a greater extent in the 5-LO<sup>-/-</sup> mice (FIG. 5 and TABLE 3). Further, significantly more new bone (mineralized tissue) was present in the 5-LO<sup>-/-</sup> fracture callus at 7 and 10 days after fracture. The data demonstrate that fracture healing is accelerated and enhanced in the 5LO-KO mice.

TABLE 2

Summary of fractured femur torsional mechanical testing data from 5-LO <sup>-/-</sup> and wild-type mice of identical genetic background and age at time of fracture (Fx).						
Mean Percentages (Fracture/Contralateral) ± SD						
Strain	Sample Size	Days Post-Fx	Peak Torque	Rigidity	Max. Shear Stress	Shear Modulus
C57BL/6	9	28	85.3 ± 16.7	61.9 ± 31.3	18.2 ± 5.4	7.8 ± 4.4
C57BL/6	6	84	77.8 ± 20.1	110.6 ± 19.1	26.2 ± 12.8	25.9 ± 13.4
Lox5 <sup>-/-</sup>	8	28	128.5 ± 30.3	109.9 ± 37.4	31.8 ± 9.0	17.4 ± 11.1
Lox5 <sup>-/-</sup>	8	84	131.4 ± 26.0	95.8 ± 37.8	49.8 ± 16.9	23.2 ± 13.2

TABLE 3

Summary of fracture callus histomorphometric analysis from 5-LO <sup>-/-</sup> and wild-type mice of identical genetic background and age at time of fracture.						
Time Point	Percent Cartilage (mean ± S.D.)			Percent Mineralized Tissue (mean ± S.D.)		
	Wild-Type	5-LO <sup>-/-</sup>	P value	Wild-Type	5-LO <sup>-/-</sup>	P value
7 days	7.84 ± 1.31	30.84 ± 3.46	<0.001	12.89 ± 3.76	24.56 ± 3.33	<0.001
10 days	20.16 ± 6.13	14.46 ± 7.53	0.226	35.49 ± 9.67	47.57 ± 2.86	0.028
14 days	3.63 ± 1.37	2.73 ± 3.71	0.625	44.66 ± 7.14	51.46 ± 5.38	0.127
21 days	0 ± 0	0 ± 0	1.000	77.26 ± 6.26	75.72 ± 2.55	0.624

The serial x-rays (FIG. 3) show that fracture healing is accelerated in the 5LO<sup>-/-</sup> mice as compared to wild type mice (C57BL/6). More specifically, the 10 day old fracture from the 5LO<sup>-/-</sup> mouse appears to be at similar stage as the 14 day old fracture from the wild type mouse, the 14 day 5LO<sup>-/-</sup> fracture is similar to the 21 day wild type fracture, and the 1 month 5LO<sup>-/-</sup> fracture is similar to a 3 month old wild type fracture. The mechanical testing data show quantitatively that the structural and material properties of the 5-LO<sup>-/-</sup> fracture callus were statistically significantly better than the controls after 4 weeks of healing with a 50% increase in peak torque, a 75% increase in rigidity, a 75% increase in maximum shear stress, and over a 100% increase in shear modulus. Further, the 4 week mechanical testing parameters from the 5LO<sup>-/-</sup> mice were similar to those from the 12 week wild type mice, supporting the x-ray data of FIG. 3 and demonstrating that fracture healing was accelerated and enhanced in the 5LO<sup>-/-</sup> mice. After 12 week of healing, the rigidity and shear modulus of the wild-type fracture callus had caught-up with the 5-LO<sup>-/-</sup> fracture callus. Histomorphometric measurements of time-staged fracture callus specimens from the 5-LO<sup>-/-</sup> and WT mice support the mechanical and radiographic observations (FIG. 5 and TABLE 2). Callus cartilage area peaked by day 7 post-fracture in the 5-LO<sup>-/-</sup> mice but not until day 10 in the WT mice. There was almost 4-times more cartilage present in the 5-LO<sup>-/-</sup> callus at day 7 as compared to that from the WT mice. Concurrently, more new bone formation also occurred in the 5-LO<sup>-/-</sup> mice with almost twice as much new bone (mineralized tissue) present at day 7 and 30% more new bone at day 10 as compared to the WT mice. The data is thus consistent with fracture healing occurring faster and producing more mechanically sound fracture callus with enhanced structural and material properties in the 5-LO<sup>-/-</sup> mice than in normal mice.

## Example 2

## COX-2 Knockout Mice

Fracture healing was assayed in mice with a targeted deletion of the COX-2 gene. Closed, mid-diaphyseal femur fractures were made in the right hindlimb of COX-2 knockout, COX-1 knockout, and wild type mice (not shown). Fracture healing was assessed by x-rays and histology (FIG. 6), and by mechanical testing (not shown). The data show that fracture healing was dramatically impaired in the COX-2 knockout mice, but not the COX-1 knockout or wild type mice. X-rays after 14 days of healing show a large mineralized fracture callus in the COX-1 knockout mouse (FIG. 6) with little or no evident mineralized callus in the COX-2 knockout mouse. Histological examination confirmed the x-ray findings in that the COX-2 knockout callus had a significant amount of cartilage but no new bone was evident. Torsional mechanical testing data shows that fracture callus structural and material properties are statistically significantly worse than COX-1 knockout or wild type mice. When combined with the experimental results of example 1, example 3, and example 4 this demonstrates how arachidonic acid metabolic or signaling can be manipulated according to the methods of the invention to affect bone formation.

## Example 3

## Treatment of Rats with a 5-Lipoxygenase Inhibitor

Sprague-Dawley rats (3 months old) underwent a standard closed femur fracture procedure as described in the art (Simon et al. Journal of Bone and Mineral Research, 17(6): 963-976 (2002); Bonnarens and Einhorn, *Production of a standard closed fracture in laboratory animal bone*. Journal of Orthopaedic Research, 2: 97-101 (1984)). The impending fracture was stabilized with an intramedullary stainless steel pin. Beginning 4 hours after fracture the rats were treated with

35

30 mg/kg of NDGA (nordihydrogairic acid) in 1% methylcellulose (5-lipoxygenase inhibitor treatment group) or with carrier only (1% methylcellulose). The day after surgery and continuing until day 14 post-fracture, experimental rats were treated with 2 doses of NDGA (30 mg/kg), the first dose between 8-10 AM and then again with another NDGA dose 8-10 hours later. Control rats were treated similarly but with carrier only (1% methylcellulose). Three weeks after fracture, the rats were sacrificed, the fractured femurs were harvested, and high resolution radiographs were made of the fractured femurs using a Packard Faxitron and Kodak MinR2000 mammography film. Two representative radiographs are shown in FIG. 7 for each treatment group: control and 5-lipoxygenase (5-LO) inhibitor treated.

The radiographs show that after 3 weeks the fractured femurs of the 5-LO inhibitor treated rats were bridged with new bone. In contrast, a well-formed, mineralized fracture callus has formed in the control rats but the fracture site had not yet bridged with new bone. In rat C, the fracture is bridged with new bone on the medial (top) and lateral (bottom) sides of the fracture callus. In rat D, the fracture is bridged with new bone on the lateral side (bottom) and shows indications of new bone bridging on the medial side. No new bone bridging is evident in the control rats (rats A and B). The data thus demonstrates that 5-LO inhibitor therapy can accelerate the fracture healing process in young, normal rats.

#### Example 4

##### Treatment of Rats with 5-Lipoxygenase Inhibitors

Sprague-Dawley rats (3 months old) underwent a standard closed femur fracture procedure as described in the art (Simon et al. *Journal of Bone and Mineral Research*, 17(6): 963-976 (2002); Bonnarens and Einhorn, *Production of a standard closed fracture in laboratory animal bone*. *Journal of Orthopaedic Research*, 2: 97-101 (1984)). The impending fracture was stabilized with an intramedullary stainless steel pin. Beginning 4 hours after fracture the rats were treated with vehicle (1% methylcellulose) or inhibitors of 5-LO suspended in 1% methylcellulose Inhibitor A (NDGA) was administered at 30 mg/kg and Inhibitor B (AA-861) was administered at 5 mg/kg. The day after surgery and continuing until day 21 post-fracture, experimental rats were treated with 2 doses of inhibitor (either A or B), the first dose between 8-LOAM and then again with another dose 8-10 hours later. Control rats were treated similarly but with carrier only (1% methylcellulose). Three weeks after fracture the rats were anesthetized and high resolution radiographs were made of the fractured femurs using a Packard Faxitron and Kodak MinR2000 mammography film (FIGS. 8A, 8B, and 8C). Five weeks after fracture the rats were sacrificed, femurs resected, and assayed for structural mechanical properties by torsional mechanical testing (FIG. 8D).

The radiographs showed that after 3 weeks of healing, the fractures appeared bridged in the 5-LO inhibitor treated rats but not in the vehicle treated rat.

Torsional mechanical testing was used to measure the peak torque sustained by each femur after 5 weeks of healing. The data show that the femurs from the Inhibitor A (NDGA) treated rats and from the Inhibitor B treated rats had 22% and 53% greater peak torque than vehicle treated rats (FIG. 8D). In addition, all of the femurs from the Inhibitor A or B treated rats failed as boney unions while 13% (2 of 15) of the femurs from the vehicle treated rats failed as non-unions with no apparent bone bridging.

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These experimental observations demonstrate that 5-LO inhibition therapy can accelerate (faster bone bridging) and enhance (better mechanical properties) fracture healing.

#### Example 5

##### Ex Vivo Treatment Methods Using Small Molecule Compounds, RNAi, and Antisense Compounds

Methods to promote ex vivo osteogenesis are used, e.g., to aid in healing of recalcitrant bone fractures, segmental defects caused by traumatic injuries or pathological resection of bone segments, or for joint arthrodesis. In these instances, precursor bone cells are isolated from a subject or from a suitable donor and are cultured ex vivo using standard methods. The cells are grown in or seeded into an appropriate scaffold that either represents the segment of missing bone or can be molded to fit the missing segment or juxtapose the ends of the bone. The cells are induced to form bone ex vivo using appropriate cell culture conditions or with inductive factors, such as bone morphogenetic protein-2 (BMP-2). Once the cells have begun to elaborate a new bone matrix, the construct can be implanted into the patient to effect osteogenesis and promote healing. This sequence of events is typically referred to as a tissue engineering approach to enhancing osteogenesis.

Inhibition of 5-lipoxygenase (5-LO) can be used to promote ex vivo bone formation for tissue engineering application. This is accomplished by promoting osteogenesis ex vivo with small molecule inhibitors of 5-LO or FLAP alone or in combination with well known inductive agents, such as BMP-2.

A second approach uses RNAi technology to inhibit 5-LO activity and promote ex vivo osteogenesis. This is accomplished by transfecting the cultured precursor skeletal cells with pools of siRNA sequences using commercially available transfection reagents, such as TransIT-TKO or jetSI. Approximately 1 million cells are transfected with a cocktail of 3 siRNAs specific for 5-LO or FLAP using 50-200 pmoles of each siRNA. Alternatively, a pool of siRNAs that target 5-LO and FLAP is used. As a control, cells are transfected similarly with commercially available siRNAs developed to knock-down enhanced green fluorescent protein (EGFP). Knock-down of 5-LO or FLAP is confirmed by western blot analysis and the results quantified to insure a greater than 80% reduction in 5-LO and/or FLAP expression.

The treated precursor skeletal cells are cultured and osteogenesis is assessed as extracellular matrix production of cartilage or bone matrix using measures such as alcian blue or alizarin red binding as appropriate or measures of specific matrix protein. Knock-down of 5-LO or FLAP promotes osteogenesis based upon enhanced calcified matrix deposition measured by alizarin red binding. This indicates that an RNAi or anti-sense approach to inhibiting 5-LO activity is useful for promoting osteogenesis ex vivo for purposes of tissue engineering.

Pools of siRNA pairs for 5-LO can be chosen, e.g., from POOL-A (5'-AAC TGG GCG AGA TCC AGC TGG-3' (SEQ ID NO: 9), 5'-AAG CTC CCG GTG ACC ACG GAG-3' (SEQ ID NO: 10), 5'-AAG GAA GCC ATG GCC CGA TTC-3') (SEQ ID NO: 11), POOL-B (5'-AAT CGA GAA GCG CAA GTA CTG-3' (SEQ ID NO: 12), 5'-AAG GAG TGG ACT TTG TTC TGA-3' (SEQ ID NO: 13), 5'-AAC TTC GGC CAG TAC GAC TGG-3') (SEQ ID NO: 14), or POOL-C (5'-AAG TTG GCC CGA GAT GAC CAA-3' (SEQ ID NO: 15), 5'-AAC ACA TCT GGT GTC TGA GGT-3' (SEQ ID NO: 16), 5'-AAC CAT GCG AGC CCC GCC ACC-3') (SEQ

ID NO: 17). Pools of siRNA pairs for FLAP can be chosen, e.g., from POOL-D (5'-AAG CAA ACA TGG ATC AAG AAA-3' (SEQ ID NO: 18), 5'-AAG TTC CTG CTG CGT TTG CTG-3' (SEQ ID NO: 19), 5'-AATTCA GCT CTT GAG AGC ATT-3' (SEQ ID NO: 20), POOL-E (5'-AAT GGA TTC TTT GCC CAT AAA-3' (SEQ ID NO: 21), 5'-AAG TAC TTT GTC GGT TAC CTA-3' (SEQ ID NO: 22), 5'-AAT CTA TTG GCC ATC TGG GCT-3' (SEQ ID NO: 23), or POOL-F (5'-AAC CAG AAC TGT GTA GAT GCG-3' (SEQ ID NO: 24), 5'-AAG TGA CTT TGA AAA CTA CAT-3' (SEQ ID NO: 25), 5'-AAT GAT GTC ATG TCA GCT CCG-3' (SEQ ID NO: 26). For brevity, only the sense strand of each siRNA pair is shown. It is well known in the art that siRNA pairs are double stranded small RNAs that have a 5'-AA overhang on the sense strand and a 5'-UU overhang on the antisense strand. It also is well known in the art that backbone chemistry modifications can be advantageous for stabilizing or improving the uptake of the siRNA molecules. Pirollo K F et al., (2003), Rait A, Sleer L S, Chang E H, "Antisense therapeutics: from theory to clinical practice," *Pharmacol Ther.* 99(1): 55-77. Manufacture of oligonucleotides with advantageous backbone chemistry modifications is within the level of ordinary skill, and use of such modified—backbone compounds (as well as non-modified-backbone compounds) is within the scope of the present invention.

One skilled in the art will recognize that in addition to direct transfection of the siRNAs into cells, expression vectors can be developed that express these or similar sequences and the expression vectors delivered to the cells by transfection, viral mediated delivery, or methods for delivering DNA molecules into cells. The expression vectors express the siRNAs leading to sustained inhibition of 5-LO, FLAP, or both and thereby promoting osteogenesis.

One skilled in the art also will recognize that additional strategies to inhibit expression of 5-LO or FLAP can be used to promote the same osteogenic effects in the precursor skeletal cells. Such technologies include use of anti-sense.

Exemplary 5-Lipoxygenase anti-sense sequences include, e.g., 5'-GCA GGT GCT TCT CGC TGC AGC C-3' (SEQ ID NO: 27), 5'-GCC AGT ACT TGC GCT TCT CG-3' (SEQ ID NO: 28), 5'-CCA TCG ATA TTG TTT TTG CC-3' (SEQ ID NO: 29), 5'-GGA GCT TCT CGG GCA GCT CTG TGC-3' (SEQ ID NO: 30), 5'-CCA GGT TCT TAT ACA GCA AGC-3' (SEQ ID NO: 31), 5'-CCA GCA GCT TGA AAA TGG GGT GC-3' (SEQ ID NO: 32), 5'-GCC CCG GGC CTT GAT GGC C-3' (SEQ ID NO: 33), 5'-CCA CGC CCT TGG CAG TCG G-3' (SEQ ID NO: 34), and 5'-GCG GAA TCG GGC CAT GGC TTC C-3' (SEQ ID NO: 35).

Exemplary FLAP anti-sense sequences include, e.g., 5'-GTT CCG GTC CTC TGG AAG CTC C-3' (SEQ ID NO: 36), 5'-CGC AGA CCA GAG CAC AGC G-3' (SEQ ID NO: 37), 5'-GCA AAC GCA GCA GGA AC-3' (SEQ ID NO: 38), 5'-CGT TTC CCA AAT ATG TAG CC-3' (SEQ ID NO: 39), 5'-GTT TTC AAA GTC ACT TCC G-3' (SEQ ID NO: 40), 5'-GGT TAA CTC AAG CTG TGA AGC-3' (SEQ ID NO: 41), 5'-GGA GCT GAC ATG ACA TC-3' (SEQ ID NO: 42), and 5'-GGC CAC GGT CAT GTT CAA GG-3' (SEQ ID NO: 43).

Thus, novel methods for promoting osteogenesis to accelerate or enhance bone fracture healing, treat bone defects, and enhance bone formation are disclosed. Although preferred embodiments of the subject invention have been described in some detail, it is understood that obvious variations can be made without departing from the spirit and the scope of the invention as defined by the appended claims.

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&lt;210&gt; SEQ ID NO 5

&lt;211&gt; LENGTH: 4465

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 5

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tgcccgcgcg tcggatgctc gcccgcgccc tgctgctgtg cgcggtcctg gcgctcagcc      180
atacagcaaa tccttgctgt tcccacccat gtcaaaaccg aggtgatgtg atgagtgtgg      240
gatttgacca gtataagtgc gattgtaccc ggacaggatt ctatggagaa aactgctcaa      300
caccggaatt ttgacaaga ataaaattat ttctgaaacc cactccaaac acagtgcact      360
acatacttac ccacttcaag ggattttgga acgttgtgaa taacattccc ttccttcgaa      420
atgcaattat gagttatgtc ttgacatcca gatcacattt gattgacagt ccaccaactt      480
acaatgctga ctatggctac aaaagctggg aagccttctc taacctctcc tattatacta      540
gagcccttcc tctgtgacct gatgattgcc cgactccctt ggggtgtcaaa ggtaaaaagc      600
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aggcagagat gatctacct cctcaagtcc ctgagcatct acggtttgct gtggggcagg      960
aggctcttgg tctggtgctt ggtctgatga tgatgccac aatctggctg cgggaacaca     1020
acagagtatg cgatgtgctt aacaggagc atcctgaatg gggatgatg cagttgttcc     1080

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aacacttgag tggetatcac ttcaactga aatttgaccc agaactactt tcaacaaac	1200
aattccagta ccaaaatcgt attgtgtgtg aatttaacac cctctatcac tggcatcccc	1260
ttctgcctga cacctttcaa attcatgacc agaaatacaa ctatcaacag tttatctaca	1320
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ttgctggcag ggttgctggt ggtaggaatg ttccaccgcg agtacagaaa gtatcacagg	1440
cttcattga ccagagcagg cagatgaaat accagtcttt taatgagtac cgcaaacgct	1500
ttatgctgaa gcctatgaa tcatttgaag aacttacagg agaaaaggaa atgtctgcag	1560
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tggactctgc ctatattttc ttacctgaac ttttgcaagt tttcaggtaa acctcagctc	3360
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aatagtatac acttatttta agtgaaaagc agagaatddd atttatagct aatdddtagct 3480
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tgactggaaa aagttacggt cccattctaa ttaatgcctt ttcttattta aaaacaaaac 3600
caaatgatat ctaagtagtt ctcagcaata ataataatga cgataaactt tctdddccac 3660
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&lt;210&gt; SEQ ID NO 6

&lt;211&gt; LENGTH: 604

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 6

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Met Leu Ala Arg Ala Leu Leu Leu Cys Ala Val Leu Ala Leu Ser His
1           5           10           15
Thr Ala Asn Pro Cys Cys Ser His Pro Cys Gln Asn Arg Gly Val Cys
20          25          30
Met Ser Val Gly Phe Asp Gln Tyr Lys Cys Asp Cys Thr Arg Thr Gly
35          40          45
Phe Tyr Gly Glu Asn Cys Ser Thr Pro Glu Phe Leu Thr Arg Ile Lys
50          55          60
Leu Phe Leu Lys Pro Thr Pro Asn Thr Val His Tyr Ile Leu Thr His
65          70          75          80
Phe Lys Gly Phe Trp Asn Val Val Asn Asn Ile Pro Phe Leu Arg Asn
85          90          95
Ala Ile Met Ser Tyr Val Leu Thr Ser Arg Ser His Leu Ile Asp Ser
100         105         110
Pro Pro Thr Tyr Asn Ala Asp Tyr Gly Tyr Lys Ser Trp Glu Ala Phe
115         120         125
Ser Asn Leu Ser Tyr Tyr Thr Arg Ala Leu Pro Pro Val Pro Asp Asp
130         135         140
Cys Pro Thr Pro Leu Gly Val Lys Gly Lys Lys Gln Leu Pro Asp Ser
145         150         155         160
Asn Glu Ile Val Glu Lys Leu Leu Leu Arg Arg Lys Phe Ile Pro Asp
165         170         175
Pro Gln Gly Ser Asn Met Met Phe Ala Phe Phe Ala Gln His Phe Thr
180         185         190

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His Gln Phe Phe Lys Thr Asp His Lys Arg Gly Pro Ala Phe Thr Asn  
 195 200 205  
 Gly Leu Gly His Gly Val Asp Leu Asn His Ile Tyr Gly Glu Thr Leu  
 210 215 220  
 Ala Arg Gln Arg Lys Leu Arg Leu Phe Lys Asp Gly Lys Met Lys Tyr  
 225 230 235 240  
 Gln Ile Ile Asp Gly Glu Met Tyr Pro Pro Thr Val Lys Asp Thr Gln  
 245 250 255  
 Ala Glu Met Ile Tyr Pro Pro Gln Val Pro Glu His Leu Arg Phe Ala  
 260 265 270  
 Val Gly Gln Glu Val Phe Gly Leu Val Pro Gly Leu Met Met Tyr Ala  
 275 280 285  
 Thr Ile Trp Leu Arg Glu His Asn Arg Val Cys Asp Val Leu Lys Gln  
 290 295 300  
 Glu His Pro Glu Trp Gly Asp Glu Gln Leu Phe Gln Thr Ser Arg Leu  
 305 310 315 320  
 Ile Leu Ile Gly Glu Thr Ile Lys Ile Val Ile Glu Asp Tyr Val Gln  
 325 330 335  
 His Leu Ser Gly Tyr His Phe Lys Leu Lys Phe Asp Pro Glu Leu Leu  
 340 345 350  
 Phe Asn Lys Gln Phe Gln Tyr Gln Asn Arg Ile Ala Ala Glu Phe Asn  
 355 360 365  
 Thr Leu Tyr His Trp His Pro Leu Leu Pro Asp Thr Phe Gln Ile His  
 370 375 380  
 Asp Gln Lys Tyr Asn Tyr Gln Gln Phe Ile Tyr Asn Asn Ser Ile Leu  
 385 390 395 400  
 Leu Glu His Gly Ile Thr Gln Phe Val Glu Ser Phe Thr Arg Gln Ile  
 405 410 415  
 Ala Gly Arg Val Ala Gly Gly Arg Asn Val Pro Pro Ala Val Gln Lys  
 420 425 430  
 Val Ser Gln Ala Ser Ile Asp Gln Ser Arg Gln Met Lys Tyr Gln Ser  
 435 440 445  
 Phe Asn Glu Tyr Arg Lys Arg Phe Met Leu Lys Pro Tyr Glu Ser Phe  
 450 455 460  
 Glu Glu Leu Thr Gly Glu Lys Glu Met Ser Ala Glu Leu Glu Ala Leu  
 465 470 475 480  
 Tyr Gly Asp Ile Asp Ala Val Glu Leu Tyr Pro Ala Leu Leu Val Glu  
 485 490 495  
 Lys Pro Arg Pro Asp Ala Ile Phe Gly Glu Thr Met Val Glu Val Gly  
 500 505 510  
 Ala Pro Phe Ser Leu Lys Gly Leu Met Gly Asn Val Ile Cys Ser Pro  
 515 520 525  
 Ala Tyr Trp Lys Pro Ser Thr Phe Gly Gly Glu Val Gly Phe Gln Ile  
 530 535 540  
 Ile Asn Thr Ala Ser Ile Gln Ser Leu Ile Cys Asn Asn Val Lys Gly  
 545 550 555 560  
 Cys Pro Phe Thr Ser Phe Ser Val Pro Asp Pro Glu Leu Ile Lys Thr  
 565 570 575  
 Val Thr Ile Asn Ala Ser Ser Ser Arg Ser Gly Leu Asp Asp Ile Asn  
 580 585 590  
 Pro Thr Val Leu Leu Lys Glu Arg Ser Thr Glu Leu  
 595 600

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<210> SEQ ID NO 7
<211> LENGTH: 5093
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

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cccagcagcc gcgccatgag ccggagtctc ttgctctggt tcttgctggt cctgctcctg    180
ctcccgcgcg tccccgtcct gctcgcggac ccagggggcg ccacgccagt gaatccctgt    240
tgttactate catgccagca ccagggcacg tgtgtccgct tcggccttga ccgctaccag    300
tgtgactgca cccgcacggg ctattccggc cccaactgca ccattccctgg cctgtggacc    360
tggctccgga attcactgcg gccacgcccc tctttcacc acttccctgct cactcacggg    420
cgctggttct gggagtgtt caatgccacc ttcattccgag agatgctcat ggcctggta    480
ctcacagtgc gctccaacct tatccccagt cccccacct acaactcagc acatgactac    540
atcagctggg agtctttctc caactgagc tattacactc gtattctgcc ctctgtgcct    600
aaagattgcc ccacaccat gggaaacaaa gggaaagaag agttgccaga tgcccagctc    660
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atgtttgcct tctttgcaca acacttcacc caccagttct tcaaaacttc tggcaagatg    780
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cgaggcatcc cgcccagag ccagatggct gtggggcagg aggtgtttgg gctgcttcc    1020
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aaggctgagc accccacctg gggcgatgag cagcttttcc agacgaaccg cctcatcctc    1140
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attgccatgg agttcaacca tctctaccac tggcaccccc tcatgcctga ctcttcaag    1320
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tcctaaggtc	caacaagaat	gcattccctg	aatctgtgcc	tgcactgaga	gggcaaggaa	2520
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&lt;210&gt; SEQ ID NO 8

&lt;211&gt; LENGTH: 599

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 8

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Met Ser Arg Ser Leu Leu Leu Trp Phe Leu Leu Phe Leu Leu Leu Leu
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Pro Pro Leu Pro Val Leu Leu Ala Asp Pro Gly Ala Pro Thr Pro Val
 20          25          30
Asn Pro Cys Cys Tyr Tyr Pro Cys Gln His Gln Gly Ile Cys Val Arg
 35          40          45
Phe Gly Leu Asp Arg Tyr Gln Cys Asp Cys Thr Arg Thr Gly Tyr Ser
 50          55          60
Gly Pro Asn Cys Thr Ile Pro Gly Leu Trp Thr Trp Leu Arg Asn Ser
 65          70          75          80
Leu Arg Pro Ser Pro Ser Phe Thr His Phe Leu Leu Thr His Gly Arg
 85          90          95
Trp Phe Trp Glu Phe Val Asn Ala Thr Phe Ile Arg Glu Met Leu Met
100          105          110
Arg Leu Val Leu Thr Val Arg Ser Asn Leu Ile Pro Ser Pro Pro Thr
115          120          125
Tyr Asn Ser Ala His Asp Tyr Ile Ser Trp Glu Ser Phe Ser Asn Val
130          135          140
Ser Tyr Tyr Thr Arg Ile Leu Pro Ser Val Pro Lys Asp Cys Pro Thr
145          150          155          160
Pro Met Gly Thr Lys Gly Lys Lys Gln Leu Pro Asp Ala Gln Leu Leu
165          170          175
Ala Arg Arg Phe Leu Leu Arg Arg Lys Phe Ile Pro Asp Pro Gln Gly
180          185          190
Thr Asn Leu Met Phe Ala Phe Phe Ala Gln His Phe Thr His Gln Phe
195          200          205
Phe Lys Thr Ser Gly Lys Met Gly Pro Gly Phe Thr Lys Ala Leu Gly
210          215          220
His Gly Val Asp Leu Gly His Ile Tyr Gly Asp Asn Leu Glu Arg Gln
225          230          235          240
Tyr Gln Leu Arg Leu Phe Lys Asp Gly Lys Leu Lys Tyr Gln Val Leu
245          250          255
Asp Gly Glu Met Tyr Pro Pro Ser Val Glu Glu Ala Pro Val Leu Met
260          265          270

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His Tyr Pro Arg Gly Ile Pro Pro Gln Ser Gln Met Ala Val Gly Gln  
 275 280 285  
 Glu Val Phe Gly Leu Leu Pro Gly Leu Met Leu Tyr Ala Thr Leu Trp  
 290 295 300  
 Leu Arg Glu His Asn Arg Val Cys Asp Leu Leu Lys Ala Glu His Pro  
 305 310 315 320  
 Thr Trp Gly Asp Glu Gln Leu Phe Gln Thr Thr Arg Leu Ile Leu Ile  
 325 330 335  
 Gly Glu Thr Ile Lys Ile Val Ile Glu Glu Tyr Val Gln Gln Leu Ser  
 340 345 350  
 Gly Tyr Phe Leu Gln Leu Lys Phe Asp Pro Glu Leu Leu Phe Gly Val  
 355 360 365  
 Gln Phe Gln Tyr Arg Asn Arg Ile Ala Met Glu Phe Asn His Leu Tyr  
 370 375 380  
 His Trp His Pro Leu Met Pro Asp Ser Phe Lys Val Gly Ser Gln Glu  
 385 390 395 400  
 Tyr Ser Tyr Glu Gln Phe Leu Phe Asn Thr Ser Met Leu Val Asp Tyr  
 405 410 415  
 Gly Val Glu Ala Leu Val Asp Ala Phe Ser Arg Gln Ile Ala Gly Arg  
 420 425 430  
 Ile Gly Gly Gly Arg Asn Met Asp His His Ile Leu His Val Ala Val  
 435 440 445  
 Asp Val Ile Arg Glu Ser Arg Glu Met Arg Leu Gln Pro Phe Asn Glu  
 450 455 460  
 Tyr Arg Lys Arg Phe Gly Met Lys Pro Tyr Thr Ser Phe Gln Glu Leu  
 465 470 475 480  
 Val Gly Glu Lys Glu Met Ala Ala Glu Leu Glu Glu Leu Tyr Gly Asp  
 485 490 495  
 Ile Asp Ala Leu Glu Phe Tyr Pro Gly Leu Leu Leu Glu Lys Cys His  
 500 505 510  
 Pro Asn Ser Ile Phe Gly Glu Ser Met Ile Glu Ile Gly Ala Pro Phe  
 515 520 525  
 Ser Leu Lys Gly Leu Leu Gly Asn Pro Ile Cys Ser Pro Glu Tyr Trp  
 530 535 540  
 Lys Pro Ser Thr Phe Gly Gly Glu Val Gly Phe Asn Ile Val Lys Thr  
 545 550 555 560  
 Ala Thr Leu Lys Lys Leu Val Cys Leu Asn Thr Lys Thr Cys Pro Tyr  
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<210> SEQ ID NO 42

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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 43

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20

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I claim:

1. A method for treating a bone fracture or a bone defect in a mammalian subject, comprising: administering to said subject a pharmaceutically effective amount of an antisense compound, wherein said antisense compound reduces the activity of cyclooxygenase-1 (COX-1).

2. The method of claim 1, wherein the condition is a bone fracture.

3. The method of claim 1, wherein the condition is a bone fracture selected from the group consisting of a non-osteoporotic fracture, an osteoporotic fracture, a fracture associated with a congenital disease, a fracture associated with an acquired disease, or an osteotomic fracture.

4. The method of claim 3, wherein the bone fracture is a non-osteoporotic fracture.

5. The method of claim 3, wherein the bone fracture is an osteoporotic fracture.

6. The method of claim 3, wherein the bone fracture is an osteotomic fracture.

7. The method of claim 1, wherein the condition is a bone defect.

8. The method of claim 1, wherein administration is in vivo.

9. The method of claim 1, wherein administration is ex vivo.

10. The method of claim 1, further comprising administering a pharmaceutically effective amount of a second compound that reduces a 5-lipoxygenase activity.

40 11. The method of claim 10, wherein the second compound reduces a 5-lipoxygenase activity by inhibiting a five lipoxygenase activating protein (FLAP).

12. The method of claim 11, wherein the second compound that reduces a 5-lipoxygenase activity comprises a small molecule.

45 13. The method of claim 12, wherein the small molecule is [(R)(+)-N'-[[5-(4-fluorophenoxy)furan-2-yl]-1-methyl-2-propynyl]-N-hydroxyurea.

14. The method of claim 12, wherein the small molecule is Abbott® ABT 761.

50 15. The method of claim 12, wherein the small molecule is selected from the group consisting of 3-[1-(4-chlorobenzyl)-3-t-butyl-thio-5-isopropylindol-2-yl]-2,2-dimethylpropanoic acid; 3-(1-(4-chlorobenzyl)-3-(1-butyl-thio)-5-(quinolin-2-yl-methoxy)-indol-2-yl)-2,2-dimethylpropanoic acid; nordihydroguaiaretic acid; 2-(12-hydroxy-dodeca-5,10-diylnyl)-3,5,6-trimethyl-1,4-benzoquinone; (N-(1-benzo(b)thien-2-ylethyl)-N-hydroxyurea); masoprocol; tenidap; flobufen; lonapalene; tagorizine; Abbott® A-121798; Abbott® A-76745; Abbott® A-78773; [(R)(+)-N'-[[5-(4-fluorophenoxy)furan-2-yl]-1-methyl-2-propynyl]-N-hydroxyurea; Abbott® ABT 761; Dainippon® AL-3264; Bayer® Bay-x-1005; Biofor® BF-389; bunaprolast; Cytomed® CMI-392; Takeda® CV-6504; enazadrem phosphate; Leo Denmark® ETH-615; flezelastine hydrochloride; Merck Frosst® L-663536; Merckle® ML-3000; 3M Pharmaceuticals® R-840; rilopirox; Schering Plough® SCH-40120;

tepopaxalin; linazolast; Zeneca® ZD-2138; Bristol-Myers Squibb® BU-4601A; carbazomycin C; lagunamycin; Wellcome® BW-70C; Ciba-Geigy® CGS-26529; Warner-Lambert® CI 1004; Warner-Lambert® PD-136005; Warner-Lambert® PD-145246; Eisai® E-3040; Fujirebio® F-1322; Fujisawa® FR-110302; Merck Frosst® L-699333; Merck Frosst® L-739010; Lilly® LY-269415; Lilly® LY-178002; Hoechst Roussel® P-8892; SmithKline Beecham® SB-202235; American Home Products® WAY-121520; American Home Products® WAY-125007; Zeneca® ZD-7717; Zeneca® ZM-216800; Zeneca® ZM-230487; 1,2-dihydro-n-(2-thiazolyl)-1-oxopyrrolo(3,2,1-kl)phenothiazine-1-carboxamide; Abbott® A-65260; Abbott® A-69412; Abbott® A-63162; American Home Products® AHR-5333; Bayer® Bay-q-1531; Boehringer Ingelheim® BI-L-357; Boehringer Ingelheim® BI-L-93BS; Boehringer Ingelheim® BIL 226XX; Bristol-Myers Squibb® BMY-30094; carbazomycin B; Wellcome® BW-B218C; Chauvin® CBS-1114; Ciba-Geigy® CGS-21595; Ciba-Geigy® CGS-22745; Ciba-Geigy® CGS-23885; Ciba-Geigy® CGS 24891; Ciba-Geigy® CGS-8515; Chiesi® CHF-1909; Warner-Lambert® CI-986; Warner-Lambert® CI-987; cirsiliol; docebenone; Eisai® E-5110; Eisai® E-6080; enofelast; epocarbazolin-A; eprovaferen; evandamine; Fisons® FPL 62064; Zeneca® ICI-211965; Zeneca® ICI-216800; Kyowa Hakko® KF-8940; Merck® L-651392; Merck® L-651896; Merck® L-652343; Merck® L-656224; Merck® L-670630; Merck® L-674636; Lilly® LY-233569; Merck® MK-591; Merck® L-655240; nitrosoxacin-A; Ono® ONO-5349; Ono® ONO-LP-219; Ono® ONO-LP-269; Warner-Lambert® PD-127443; Purdue Frederick® PF-5901; Rhone-Poulenc Rorer® Rev-5367; Rhone-Poulenc Rorer® RG-5901-A; Rhone-Poulenc Rorer® RG-6866; Roussel-Uclaf® RU-46057; Searle® SC-41661A; Searle® SC-45662; Sandoz® SDZ-210-610; SmithKline Beecham® SK&F-104351; SmithKline Beecham® SK&F-104493; SmithKline Beecham® SK&F-105809; Synthelabo® SL-81-0433; Teijin® TEI-8005; Terumo® TMK-777; Terumo® TMK-781; Terumo® TMK-789; Terumo® TMK-919; Terumo® TMK-992; Teikoku Hormone® TZI-41127; American Home Products® WAY-120739; American Home Products® WY-47288; American Home Products® WY-48252; American Home Products® WY-50295; Yoshitomi® Y-19432; 4-{3-[4-(2-methyl-1H-imidazol-1-yl)phenylthio]}phenyl-3,4,5,6-tetrahydro-2H-pyran-4-carboxamide; esculetin; phenidone; Boehringer Ingelheim® BI-L-239; 5,8,11-eicosatriynoic acid; 5,8,11,14-eicosatetraynoic acid; cinnamyl-3,4-dihydroxy-alpha-cyanocinnamate; curcumin; gossypol; caffeic acid; baicalein; 7,7-dimethyleicosadrenoic acid; Lilly® LY-311727; bromoenol lactone; methyl arachidonyl fluorophosphonate; methyl y-linolenyl fluorophosphonate; oleyoxyethyl phosphorylcholine; arachidonyl trifluoromethyl ketone; n-(p-aminocinnamoyl)anthranilic acid; mepacrine; quinacrine; atabrine; parabromophenacylbromide; aristolochic acid; cortisone; Glaxo SmithKline® SB-480848; Glaxo SmithKline® SB-659032; Glaxo SmithKline® SB-677116; Bristol-Myers Squibb® BMS-181162; Sterling-Winthrop® MJ33; and Millennium Pharmaceuticals® MLN977.

16. The method of claim 12, wherein the small molecule is selected from the group consisting of masoprocol; tenidap; (N-(1-benzo(b)thien-2-ylethyl)-N-hydroxyurea); flobufen; lonapalene; tagorizine; 2-(12-hydroxydodeca-5,10-diynyl)-3,5,6-trimethyl-1,4-benzoquinone; Abbott® A-121798; Abbott® A-76745; Abbott® A-78773; [(R)(+)-N'-[[5-(4-fluorophenoxy)furan-2-yl]-1-methyl-2-propynyl]-N-hydroxyurea; Abbott® ABT 761; Dainippon® AL-3264; Bayer® Bay-x-1005; Biofor® BF-389; bunaprolast; Cytomed®

CMI-392; Takeda® CV-6504; Ciba-Geigy® CGS-26529; enazadrem phosphate; Leo Denmark® ETH-615; flezelastine hydrochloride; Merck Frosst® L-663536; Merck Frosst® L-699333; Merckle® ML-3000, 3M Pharmaceuticals® R-840; rilopirox; Schering Plough® SCH-40120; tepoxalin; linazolast; Zeneca® ZD-7717; Zeneca® ZM-216800; Zeneca® ZM-230487; Zeneca® ZD-2138; and nordihydroguaiaretic acid.

17. The method of claim 12, wherein the small molecule is selected from the group consisting of tenidap; (N-(1-benzo(b)thien-2-ylethyl)-N-hydroxyurea); flobufen; lonapalene; tagorizine; 2-(12-hydroxydodeca-5,10-diynyl)-3,5,6-trimethyl-1,4-benzoquinone; Abbott® A-121798; Abbott® A-76745; Abbott® A-78773; [(R)(+)-N'-[[5-(4-fluorophenoxy)furan-2-yl]-1-methyl-2-propynyl]-N-hydroxyurea; Abbott® ABT 761; Ciba-Geigy® CGS-26529; Biofor® BF-389; Cytomed® CMI-392; Leo Denmark® ETH-615; Merck Frosst® L 699333; Merckle® ML-3000; 3M Pharmaceuticals® R-840; linazolast; Zeneca® ZD-7717; Zeneca® ZM-216800; Zeneca® ZM-230487; Zeneca® ZD-2138, and nordihydroguaiaretic acid.

18. The method of claim 11, wherein the second compound that reduces a 5-lipoxygenase activity comprises a nucleic acid comprising a sequence selected from the group consisting of 5'-AAC TGG GCG AGA TCC AGC TGG-3' (SEQ ID NO: 9), 5'-AAG CTC CCG GTG ACC ACG GAG-3' (SEQ ID NO: 10), 5'-AAG GAA GCC ATG GCC CGA TTC-3' (SEQ ID NO: 11), 5'-AAT CGA GAA GCG CAA GTA CTG-3' (SEQ ID NO: 12), 5'-AAG GAG TGG ACT TTG TTC TGA-3' (SEQ ID NO: 13), 5'-AAC TTC GGC CAG TAC GAC TGG-3' (SEQ ID NO: 14), 5'-AAG TTG GCC CGA GAT GAC CAA-3' (SEQ ID NO: 15), 5'-AAC ACA TCT GGT GTC TGA GGT-3' (SEQ ID NO: 16), 5'-AAC CAT GCG AGC CCC GCC ACC-3' (SEQ ID NO: 17), 5'-AAG CAA ACA TGG ATC AAG AAA-3' (SEQ ID NO: 18), 5'-AAG TTC CTG CTG TTG CTG-3' (SEQ ID NO: 19), 5'-AAT TCA GCT CTT GAG AGC ATT-3' (SEQ ID NO: 20), 5'-AAT GGA TTC TTT GCC CAT AAA-3' (SEQ ID NO: 21), 5'-AAG TAC TTT GTC GGT TAC CTA-3' (SEQ ID NO: 22), 5'-AAT CTA TTG GCC ATC TGG GCT-3' (SEQ ID NO: 23), 5'-AAC CAG AAC TGT GTA GAT GCG-3' (SEQ ID NO: 24) 5'-AAG TGA CTT TGA AAA CTA CAT-3' (SEQ ID NO: 25), and 5'-AAT GAT GTC ATG TCA GCT CCG-3' (SEQ ID NO: 26).

19. The method of claim 10, wherein the second compound that reduces a 5-lipoxygenase activity comprises a nucleic acid comprising a sequence selected from the group consisting of 5'-GCA GGT GCT TCT CGC TGC AGC C-3' (SEQ ID NO: 27), 5'-GCC AGT ACT TGC GCT TCT CG-3' (SEQ ID NO: 28), 5'-CCA TCG ATA TTG TTT TTG CC-3' (SEQ ID NO: 29), 5'-GGA GCT TCT CGG GCA GCT CTG TGC-3' (SEQ ID NO: 30), 5'-CCA GGT TCT TAT ACA GCA AGC-3' (SEQ ID NO: 31), 5'-CCA GCA GCT TGA AAA TGG GGT GC-3' (SEQ ID NO: 32), 5'-GCC CCG GGC CTT GAT GGC C-3' (SEQ ID NO: 33), 5'-CCA CGC CCT TGG CAG TCG G-3' (SEQ ID NO: 34), 5'-GCG GAA TCG GGC CAT GGC TTC C-3' (SEQ ID NO: 35), 5'-GTT CCG GTC CTC TGG AAG CTC C-3' (SEQ ID NO: 36), 5'-CGC AGA CCA GAG CAC AGC G-3' (SEQ ID NO: 37), 5'-GCA AAC GCA GCA GGA AC-3' (SEQ ID NO: 38), 5'-CGT TTC CCA AAT ATG TAG CC-3' (SEQ ID NO: 39), 5'-GTT TTC AAA GTC ACT TCC G-3' (SEQ ID NO: 40), 5'-GGT TAA CTC AAG CTG TGA AGC-3' (SEQ ID NO: 41), 5'-GGA GCT GAC ATG ACA TC-3' (SEQ ID NO: 42), and 5'-GGC CAC GGT CAT GTT CAA GG-3' (SEQ ID NO: 43).

20. The method of claim 1, further comprising administering to the subject a pharmaceutically effective amount of a small molecule compound that reduces a COX-1 activity.

21. The method of claim 20, wherein the small molecule compound that reduces the COX-1 activity is selected from the group consisting of Searle® SC-560, 1-[(4,5-bis(4-methoxyphenyl)-2-thiazoyl)carbonyl]-4-methylpiperazine hydrochloride, valeryl salicylate, aspirin, dexketoprofen, keterolac, flurbiprofen, and suprofen. 5

22. The method of claim 1, further comprising administering to the subject a pharmaceutically effective amount of a third compound that increases a COX-2 activity. 10

23. The method of claim 22, wherein the third compound is selected from the group consisting of prostaglandin E; butaprost; sulprostone; Pfizer® CP-536,745-01; Pfizer® CP-043, 305-02; Pfizer® CP-044,519-02; Pfizer® CP432; Ono Pharmaceutical® ONO-4819; Pfizer® CP-533,536; prostaglandin F<sub>2α</sub>; bimatoprost; cloprostenol; latanoprost; tafluprost; bone morphogenetic protein-2; platelet derived growth factor; interleukin-1α; interleukin-1β; tumor necrosis factor-α; fibroblast growth factor, transforming growth factor-β; epidermal growth factor; parathyroid hormone; parathyroid hormone related peptide; and teriparatide. 15 20

24. The method of claim 1, further comprising administering to the subject an ultrasound therapy or exposing the subject to a pulsed electromagnetic field in an amount sufficient to increase a COX-2 activity in the subject. 25

\* \* \* \* \*

UNITED STATES PATENT AND TRADEMARK OFFICE  
**CERTIFICATE OF CORRECTION**

PATENT NO. : 8,980,851 B2  
APPLICATION NO. : 12/940995  
DATED : March 17, 2015  
INVENTOR(S) : James Patrick O'Connor

Page 1 of 1

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the Claims:

Column 75, line 20, in Claim 23, replace "interleukin-1a;" with -- interleukin-1 $\alpha$ ; --.

Signed and Sealed this  
Third Day of May, 2016



Michelle K. Lee  
*Director of the United States Patent and Trademark Office*